Congress of the United States
Office of Technology Assessment

NEW DEVELOPMENTS IN BIOTECHNOLOGY
FIELD-TESTING ENGINEERED ORGANISMS: GENETIC AND ECOLOGICAL ISSUES

Contractor Documents, Volume 2

March 1988

Norse, Elliott A. 1986. ***Ecological Issues Relevant to Environmental Applications of Genetically Altered Organisms.*** Including:

Sharples, Francis E. ***Relevance of Lessons from Introductions of Exotic Species.***

Pimm, Stuart L, and Bruce R. Levin. ***Impact on Competitive Abilities of Specific Genetic Alterations.***

Keeler, Kathleen, and Andrew P. Dobson. ***The Big and Small of It: Are There Different Risks to Releasing Genetically Engineered Macro-organisms and Micro-organisms?***

Lenski, Richard E. and Conrad A. Istock. ***Are There Specific Genetic Modifications That Are Inherently So Safe as to Need No Review?***

Coleman, David C. and Robert E. Hodson. 1987. ***An Ecosystems Approach to Potential Perturbations of Energy Flow and Nutrient Cycles Associated with Environmental Applications of Genetically Altered Organisms.***

Gosz, James R., C. N. Dahm, and Patrick W. Flanagan. 1987. ***Ecological Impact of Genetically Engineered Organisms on Ecosystems.***

Massey, Adrianne, and Fred Gould. 1987. ***The Genetic Basis of Changes in Host Range or Habitat.***

These are contractor documents used in preparing OTA's final Special Report. OTA makes these papers available for the use of readers desiring a more detailed or technical discussion of an issue than can normally be accommodated in a final report. As OTA contractor documents, they have not been reviewed or approved by the Technology Assessment Board. The findings and conclusions expressed are those of the authors and do not necessarily reflect the views of OTA, the Advisory Panel or the Technology Assessment Board.

J952-20

This document can be ordered in either paper or microfiche format from:

National Technical Information Service
U.S. Department of Commerce
5285 Port Royal Road
Springfield, Virginia 22161
Telephone: (703) 487-4650
Telex: 89-9405

ECOLOGICAL ISSUES RELEVANT TO ENVIRONMENTAL APPLICATION OF GENETICALLY ENGINEERED ORGANISMS

PREPARED FOR THE OFFICE OF TECHNOLOGY ASSESSMENT, CONGRESS OF THE UNITED STATES

by

Andrew P. Dobson
Conrad A. Istock
Kathleen H. Keeler
Richard E. Lenski
Bruce R. Levin
Elliott A. Norse, Coordinator
Stuart L. Pimm
Frances E. Sharples

December 22, 1986

CONTENTS

Page

1 "Introduction." Elliott A. Norse, Public Affairs Office, The Ecological Society of America*

3 "Executive Summary." Elliott A. Norse

6 "Relevance of Lessons From Introductions of Exotic Species." Frances E. Sharples, Environmental Sciences Division, Oak Ridge National Laboratory

16 "Impact on Competitive Abilities of Specific Genetic Alterations." Stuart L. Pimm, Department of Zoology, University of Tennessee and Bruce R. Levin, Department of Zoology, University of Massachusetts

31 "The Big and Small of It: Are There Different Risks to Releasing Genetically Engineered Macro-organisms and Micro-organisms?" Kathleen H. Keeler, School of Biological Sciences, University of Nebraska--Lincoln and Andrew P. Dobson, Department of Biology, University of Rochester

53 "Are There Specific Genetic Modifications That Are Inherently So Safe as to Need No Review?" Richard E. Lenski, Department of Ecology and Evolutionary Biology, University of California--Irvine and Conrad A. Istock, Department of Ecology and Evolutionary Biology, University of Arizona

* now affiliated with The Wilderness Society, 1400 I Street, N.W., Washington, D.C. 20005.

INTRODUCTION

Elliott A. Norse
Public Affairs Office
The Ecological Society of America

In the last dozen years, as molecular biologists have made quantum advances in their ability to alter the genetic code, scientific concerns have arisen about possible adverse consequences of genetic engineering. The first to voice such concerns were molecular biologists who feared that microorganisms with new characteristics might inadvertently escape from the laboratory and threaten public health. These scientists encouraged the federal government to emplace safeguards that appear to have been remarkably effective, allowing them to continue to expand their research while preventing these organisms from entering the environment.

More recently, as researchers in academia and the infant biotechnology industry have sought to introduce engineered organisms into the environment deliberately, rather than confining them, scientific concerns have arisen from another quarter--ecologists and evolutionary biologists. These scientists study the interrelations of organisms and their environments as they vary in space and time, and have observed the consequences of natural and deliberate introductions of organisms into ecosystems where they did not previously occur.

It should not be surprising that scientists who work and gain their insights in the controlled conditions of the laboratory or the highly simplified conditions of the agronomic test plot see things differently from scientists who work and gain their insights in the vastly more complex natural world. The former eliminate all possible sources of variance as extraneous so that they might understand the workings of the individual components of living systems. The latter have resigned themselves to studying the spatial and temporal biological dynamics of a world in which many variables are uncontrolled. Experience has led many of the former to believe that genetically engineered organisms are inherently harmless or pose, at most, a minimal risk. Experience has led many of the latter to question that conclusion.

One thing common to both groups is their enthusiasm for the benefits that genetic engineering could bring. No informed body of biologists doubts the potential of genetic engineering for treating disease, improving food production or eliminating environmental pollutants. More specifically, as one who has spoken to many colleagues about this issue in recent years, I know of no ecologists or evolutionary biologists who are opposed to genetic engineering or the use of engineered organisms in the environment. Indeed, a growing number see genetic engineering as a potent new tool for their research. Rather, many of my colleagues are concerned that, in the absence of suitable safeguards and study, these benefits could be obtained at too great a price.

At the request of OTA, I have assembled seven distinguished ecologists and evolutionary biologists representing different subdisciplines and institutions to examine four questions central to assessing the safety of environmental application of genetically engineered organisms. The time available for producing this report was very short. Although I endeavored to make the formats of the papers consistent with one another, corrected a few typographical errors and questioned the authors about some points in the

drafts, I did not edit the substance of these papers; they are the authors' unabridged thoughts. Under these circumstances, I find the strength and unity of their logic to be a remarkable reflection on the vitality of ecology and evolutionary biology and their potential for advancing the environmental application of genetically engineered organisms.

I hope that these papers will help genetic engineers understand the scientific basis for concern about current regulations and help decisionmakers with the difficult task of balancing the growth of a promising new industry with the well-being of the public and our environment.

EXECUTIVE SUMMARY

Elliott A. Norse
Public Affairs Office
The Ecological Society of America

This study by seven ecologists and evolutionary biologists examines four questions central to assessing the safety of releasing genetically engineered organisms into the environment.

Sharples examines the relevance of lessons from introductions of exotic (alien) species to environments in which they did not occur to introductions of genetically engineered organisms. She points out that many thousands of species have been spread from their native homes, either intentionally or accidentally. Environments that are naturally biologically simple or that have been simplified by humans--oceanic islands, physically disturbed or chemically polluted ecosystems, agricultural systems--are particularly vulnerable to invasion. Most successful introductions are organisms that 1) are very generalized in their requirements and tolerances; 2) have adaptations predisposing them to survival in the ecosystems to which they are introduced; or 3) have unique methods of gaining access to needed resources. Introduced organisms exert ecological effects through predation (including herbivory), competition, disease, parasitism, by interfering with existing mutualisms, and by hybridization.

Sharples explains the strong analogy between exotic and genetically engineered organisms. The genetic differences between organisms need not be large to have profound adverse effects; single gene mutations can be sufficient. Further, convergent evolution demonstrates that knowing the genotype of an organism does not permit one to predict the phenotypic expression of the genes in an ecological context. Thus, it is not the amount of genetic differences, but their ecological expression that is important. This is especially relevant to biotechnology, which might make only quantitatively small alterations that are, nonetheless, specifically designed to produce major differences in the ecology of the organisms. Although some general risk factors are known, risks of introducing specific engineered organisms can be calculated much more precisely from intensive, species-focused field and laboratory ecological research.

Pimm and Levin examine the assertion that genetic alterations lower fitness of organisms, and therefore prevent genetically modified introductions from having adverse consequences. They point out that outcrossing and genetic recombination in bacteria, unlike that of plants and animals, is an infectious process in which accessory elements and possibly chromosomal genes are transferred among the same and different species. There is no justification for the general assertion that genetic alterations will always lower fitness, although less generalized statements might be justified. It is useful to consider three groups of genetic alterations: 1) simple mutations, i.e. small substitutions, additions or deletions of a few bases; 2) reorganizations, which are large-scale changes that are more likely to have more profound and encompassing effects; and 3) accessory elements. In theory, alterations of coadapted gene complexes should usually lower fitness, but the evidence for simple mutations, reorganizations and accessory elements is equivocal. On a case-by-case basis, it may be possible to ascertain whether a specific

alteration would be selected for if there were sufficient information about the phenotype coded for and the environment into which the organism would be released. However, the ways that genetic background and the environment affect expression of genetic modifications limit predictability about their ecological consequences. There is no justification for assuming that selection will favor recombinants generated by exchanges from closely or distantly related species.

Pimm and Levin also explain that organisms with severe genetic handicaps can survive long enough to be selected for. A mutant that is inefficient but able to exploit an abundant but unused resource may fare better than a wild type that efficiently uses a depleted resource. Introductions are most successful in habitats modified by humans, and usually have a negative effect on other species. Impacts are likely to be most severe where predators are introduced where they had been absent; when polyphagous (generalized feeders) organisms are introduced; and where organisms are introduced into systems with a few plant species whose loss can cause the collapse of entire food chains. These patterns have been frequently observed in introductions of organisms genetically modified by humans.

Keeler and Dobson examine the degree to which ecological insights about macroorganisms can be applied to microorganisms, and vice-versa. They point out that the public fears potentially dangerous microorganisms far more than macroorganisms. Microorganisms can have major effects by causing disease in people or living things we value, or by disrupting essential ecosystem processes, such as the nitrogen cycle. Most reported cases of competition between introduced and indigenous organisms have not resulted in extinctions, but competition is most likely to be a serious consequence when the introduced organism is most similar to indigenous organisms, as when a small fraction of the genome is modified. Hence, slightly modified organisms should receive special scrutiny. Superior competitors might eliminate indigenous organisms directly, or by interfering with mutualistic relationships essential to indigenous organisms. There should be no difference in the impact of competition on macro- and microorganisms; in fact, available ecological models fit microorganisms better. The spread of pathogens is particularly well understood through ecological modeling, and design of pathogens as biological control agents forms a productive area for cooperation between population and molecular biologists. To minimize adverse environmental consequences, it is essential that pathogens be highly specific for their intended hosts, and it might be advisable to mandate limits on the reproductive rate of engineered pathogens. One of the more serious problems is the possibility of cascade effects, i.e. changes in one species that destabilize other species, leading to changes in species far removed from the original disturbance. For example, inoculation of cattle against the rinderpest virus, which also affects wild hoofed mammals, led to increases in lions and hyenas but decreases in hunting dogs. Such changes can be counter-intuitive and completely unpredictable.

Keeler and Dobson explain that major differences between macroorganisms and microorganisms are a consequence of their different sizes. Microorganisms are much more likely to mutate and speciate than macroorganisms, partly because their populations or application rates are so much higher. Hence, evolutionary lability should be a major consideration in deliberate releases. Not only are microorganisms more capable of evolutionary change; they are more likely to slip into ecosystems unnoticed. Since far more is known about macroorganisms,

cooperation between microbiologists and population ecologists is essential to fulfilling the potential of biotechnology.

Lenski and Istock examine whether there are any genetic modifications inherently so safe as to need no pre-release review in light of the Administration's "Coordinated Framework for Regulation of Biotechnology." They point out that engineered organisms can be viewed as inherently safe if they will not survive or reproduce after their release, their functions have no lasting effect, and the engineered genes will not be transferred to other organisms. Crop plants and domesticated animals are likely to be safe if the there is no gene flow to related wild organisms, the organisms have no damaging weedy or feral relatives, and they do not substantially alter ecological processes such as nitrogen cycling. Modified known pathogens and pests merit detailed scrutiny whether or not they are outright or opportunistic problems. The distinction between outright and opportunistic pathogens is arbitrary and subject to change; once-opportunistic organisms have become major disease or pest organisms as a result of human environmental modifications. The difficulties of classifying organisms, particularly microorganisms, makes the distinction between intergeneric and intrageneric transfers ill-advised. Because intrageneric variants are less novel, they are less likely to disrupt coadapted gene complexes, so such organisms are more likely to persist.

Lenski and Istock take strong exception to exclusion of altered non-coding sequences and deletions. Major genetic differences (e.g., 50-fold increases in tetracycline resistance in bacteria and expression of Down's syndrome in humans) can result without altering coding sequences. Regulatory sequences that determine gene dosage and timing, thereby affecting organismic structure and function, have major influence on ecological and evolutionary relationships. Organisms modified by deletion may have important impacts if they are released in large numbers. They also stress that non-essential genetic materials should be excised from recombinants; there is no justification for releasing organisms with antibiotic resistance markers. In general, unintended pathogenicity is not the only possible adverse impact of environmental release, unpredictable ecological effects can arise from perturbations that are not highly novel, and products or processes that are safe at one level or scale may be unsafe at another. As a result, evaluation of risks should proceed on a case-by-case basis.

RELEVANCE OF LESSONS FROM INTRODUCTIONS OF EXOTIC SPECIES

Frances E. Sharples
Environmental Sciences Division
Oak Ridge National Laboratory

In the debate on the safety of genetically engineered organisms, ecologists have repeatedly asserted that uncontained uses of such organisms in the environment are a cause for concern and require evaluation of risks. These assertions are largely based on a considerable body of information documenting negative ecological effects from introductions of non-engineered organisms into ecological communities where they did not previously occur. This section will provide a brief overview of what is known about the dynamics and effects of species introductions, and a discussion of why the results of exotic introduction are relevant to the subject of potential environmental effects of genetically engineered organisms.

What Are the Lessons? A Brief Overview

The number of times plant, animal, and microorganism species have been spread from their native homes, either purposefully or accidentally, to new environments almost certainly numbers in the tens of thousands. For example, Ebenhard (in press) identified from the literature a total of 1559 incidents world-wide of successful (self-propagating) establishment just of bird and mammal species. In North America alone, 150 species of freshwater fish have been established outside their historical ranges, over 150 species of introduced marine invertebrates are resident on the Pacific coast (Moyle, 1986), and roughly 1550 foreign insect species are successfully established in the United States (Simberloff, 1986). Still, no one really knows how many times introductions have been attempted and have failed, because in most instances failed introductions go unrecorded and unnoticed. What is certain is that the number of successful establishments represents an unknown fraction, which is probably small, of the total number of introduction attempts that have occurred. Given this fact, ecologists have been endeavoring to understand the factors that allow success of exotic invasions for decades. Efforts to answer questions about how communities of living organisms are "assembled" and what controls the number of species found in particular environments are central to the study of ecology.

As in any science, ecology seeks to detect patterns in natural systems and to explain the causal processes that produce these patterns. But ecological systems are extremely complex, and this complexity may be difficult to work around. In other sciences, a hypothesis can usually be tested by experiment. In ecology, experiments may not help because small scale experiments may say little about large scale processes, and the deliberate production of serious ecosystemwide perturbations for the purposes of large scale experiments would be unconscionable. Furthermore, for the majority of exotic introductions, ecologists are forced to evaluate events that have happened well back in the past where there can be no hope of anything that approximates controlled experimental design. A number of generalizations about the characteristics of vulnerable environments and successful invading species have nevertheless emerged from the study of past experiences. Although there is currently some controversy over the value to be

placed on such generalizations, it is still true that they fit the empirical observations of a great many investigators.

What Kinds of Environments are Most Vulnerable to Invasion?

Perhaps the single best known effort to answer this question was by the British ecologist Charles Elton who published an early synthesis of knowledge in a fascinating and classic book called "The Ecology of Invasions by Animals and Plants" (Elton, 1958). Elton's little book contained an eloquent plea for the conservation of natural variety. Having examined a large number of cases of ecological dislocation produced by introduced species, he argued that their effects were more frequent and more potent in certain kinds of simplified environments, where the simplification might be natural or due to human interference. Such environments include man-made communities (e.g., agricultural systems) in which there are very few species by design; stressed or disturbed systems that have been polluted or subjected to repeated physical damage; and islands, especially oceanic islands, where native species diversities are low because of geographical remoteness and the difficulty organisms face in dispersing there. Elton believed that ecological systems that are more complex are also more stable and argued that it is to our advantage to preserve natural complexity to help nature help us avoid the damage caused by "ecological explosions" of introduced species.

Elton's argument and analysis were very simple and based on his own astute observations, but other great theoretical ecologists, including G. Evelyn Hutchinson, Robert MacArthur, and Ramon Margalef, were also lead to the same conclusion. The premise that complexity produces stability in communities became an early central tenet of ecology. Although this idea lost favor as ecological theory became increasingly involved with mathematical modeling, it has recently re-emerged with the realization that discrepancies between model behavior and empirical observation were partly due to different ecologists using the word "stability" to mean many different things. Contemporary investigators are now returning to the concept in greatly refined form. Pimm (1984, 1986), for example, clarifies stability by defining four additional concepts: resilience, persistence, resistance, and variability. Each of these describes aspects of stability, and deals with the behavior of an ecological system returning to equilibrium following a perturbation. In these terms, for example, the introduction of a new species is a question of the persistence of the invaded community--how long its composition remains stable before the introduction is successful and changes that composition--and its resistance--how much the community is changed by an introduction. Both improved models (e.g., Post and Pimm, 1983) and empirical studies are once again suggesting that communities with few species are more vulnerable to invasion. Recent examples include Ebenhard (in press) who found that of the 1559 establishments of birds and mammals he evaluated, roughly 20% had occurred on continents, 20% on continental shelf islands, but 60% occurred on isolated oceanic islands. Similarly, Crawley (1986) found that invasibility of plant communities in Britain reflected frequency and intensity of disturbance, with alien plants making up more than 50% of the floras of urban waste ground but less than 5% of unmanaged native woodland floras. Finally, Moyle (1986) documents that exotics typically constitute less than 10% of fish populations in North America east of the Rocky Mountains where native fish faunas are naturally rich and complex.

West of the Rockies, where river drainages are highly distinct and separate and contain relatively small numbers of native species, exotics make up 30 to 60% of total fish faunas.

Does Species Richness Prevent Successful Invasion?

Another way to handle the question of which environments are more or less vulnerable to establishment of an added species is to compare patterns in "species-rich" vs "species-poor" communities. Diamond and Case (1986), for example, reviewed data on worldwide bird introductions in which they detected interesting patterns.

A. Invasion success declines with species "richness", S, or the number of species extant in an invaded fauna.

For Ascension Island, with no native bird species extant, invasion success was 90%. For Lord Howe, Bermuda, Rodriguez, and the Seychelles, invasion success was 60 to 80%, with S varying from 3 to 17. For Borneo (S about 420) and New Guinea (S = 513), invasion success was 0%.

B. The penetration of native forest declines with species richness of the native avifauna.

On Viti Levu, the largest island of the Fiji archipelago, introduced species are strictly confined to man-modified habitats (S = 48). On Oahu (S=10), introduced birds are abundant in the native forest, and on Kauai (S=19), the number of exotics penetrating native forest is intermediate between Viti Levu and Oahu. And some species that penetrate native forest on species-poor islands do not penetrate on species-rich ones. Diamond and Case also indicate that data on other introduced vertebrates conform to these same patterns, though this analysis has not yet been published.

What Kinds of Organisms Make Successful Invaders?

In general, characteristics that contribute to the success of invaders fall into three loose categories.

A. Established exotics are frequently very generalized in their requirements and tolerances.

This is to say that they are often habitat and/or food generalists and have high reproductive rates and dispersal capabilities. All of these factors are generally related to an ability to be flexible and opportunistic, taking advantage of whatever resources are available. A few examples will illustrate.

The notorious gypsy moth can feed on 450 to 500 different plant species, is highly fecund, and has proved itself able to adapt behaviorally to adverse climatic conditions once thought likely to furnish limits to its spread. The mongoose was introduced into many Caribbean and Pacific islands in the late 19th century to control introduced rats and has been blamed for all manner of destructive effects including extinctions of native birds and reptiles. It is an aggressive and omnivorous predator with very generalized habitat requirements, and is also highly fecund, producing 2 litters of 2-4 pups over a

long breeding season each year. Finally, Dritschilo et al. (1985) analyzed data on a total of 1263 established introduced species (mostly plants and insects) in California. They concluded that if one could characterize such a species, the following descriptions would be appropriate. Successful insects can feed on a variety of foods, survive in a varied range of habitat types, and have very high reproductive capacities. A similar description would apply to successful exotic plants.

B. Preadaptation of an exotic may contribute greatly to successful establishment.

If an organism is introduced into an environment with characteristics that match those of the environment in which it evolved in the first place, it may be predisposed for success. It is no coincidence that most of the many introduced species in California originated in areas with Mediterranean climates similar to that of California. Similarly, although Europe is further away from the United States than Central and South America are, 66% of the exotic insects in this country are of European origin. Only 14% are Latin American (Simberloff, 1986), suggesting that insects adapted to European conditions find the climate, crops, and overall vegetation types of the United States more hospitable.

C. Successful invaders frequently have unique methods of gaining access to resources they need.

Such a property may make an invader more efficient at gathering resources than resident organisms are, may allow use of resources inaccessible to residents, or may represent a "style" of resource acquisition which native species are unprepared to cope with. Again, a couple of examples will illustrate. Introduced salt cedar (Tamarix sp.) is a deep-rooting plant with a rapid transpiration rate. Although it requires wet conditions to germinate, once established it is able to tap water that is deep in the soil and unavailable to other plants. In the arid and semiarid southwest, it invades desert water courses where its rapid transpiration results in drying up of natural springs to the detriment of other desert inhabitants (Vitousek, 1985).

Unfamiliar styles of predation are often significant in enabling introduced predators to devastate native prey populations. The sea lamprey's unusual mode of predatory attack (attachment to a fish, then rasping and sucking the flesh until death occurs) is believed to have contributed greatly to its ability to virtually wipe out the larger native fish in Lake Michigan (Moyle, 1986).

Finally, in microorganisms, genes for degradation of unusual compounds, such as xenobiotics, may allow use of a resource for which there is little or no competition from other organisms. The ability to take advantage of such a resource may allow an introduced microorganism to persist long enough to undergo selection for increased efficiency and improved survival. (See the discussion of the "adaptive peak" metaphor in the section by Pimm and Levin.)

Effects of Introduced Organisms

In general, when an added organism is said to have produced an ecological effect, what is usually meant is that changes in the distribution and abundance of one or more resident species in an ecosystem have occurred. In the most extreme case, it may mean that the extinction of one or more native species has resulted. Experimental studies show that few natural communities are "species-deletion stable" (Pimm, 1986); that is, the loss of one species causes further losses of other species. And the reduction or loss of one or more species may, in turn, result in changes in the dynamics of energy flow and material cycling through the system.

Most authors recognize roughly six categories of mechanisms by which ecological effects may be exerted. Diamond and Case (1986), for example, identify predation, competition, herbivory, disease, parasitism, and mutualism. The last category refers to second order effects where the survival of one species is threatened by the disappearance of another species on which it is dependent (e.g., for pollination). Similarly, Ebenhard (in press) recognizes herbivory, predation, competition, parasitism/pathogenicity, and genetic effects through hybridization. The last refers to the disappearance of distinct endemic species or subspecies by genetic overwhelming via hybridization with introduced close relatives. Depending on the type of organism, most, if not all, of these effects mechanisms could be relevant to organisms that are engineered and released.

Why the Lessons Are Relevant

Most ecologists and evolutionary biologists view environmental uses of genetically engineered organisms as an extension of subset of introduced species problems (e.g., Alexander, 1985; Dritschilo, et al., 1985; Regal, 1986). The "introduced species model" seems to represent the best of a number of frameworks for thinking about the potential for unwanted ecological effects following release. This section will provide a brief discussion of the reasons for this view.

Experience with Small Genetic Changes

The argument is frequently made that the small amounts of genetic information typically transferred between species in genetic engineering constitute so small a portion of the genome that comparing such modified organisms to an exotic species is inappropriate. There is considerable evidence, however, that small genetic changes are frequently involved in drastic modifications of the relationships of organisms in their environments. The most direct evidence comes from two areas of study: development of resistance to chemical control agents and host/pathogen or parasite population dynamics.

The development of resistance is well illustrated for antibiotics and insecticides. When antibiotics were first introduced, they were extremely effective at killing bacteria. Initially sensitive strains have, however, rapidly and repeatedly become resistant to each new antibiotic with great clinical significance. Many problems in the treatment of bacterial illnesses have arisen with serious consequences. Hospital acquired infections are, for example, notoriously dangerous and difficult to treat because of multiple resistance traits which limit the number of drugs that remain effective. Major epidemics due to multiply-resistant pathogens have also occurred, often with

extremely high fatality rates. In 1969-70, for example, a major epidemic of bacillary dysentery occurred in Mexico and Central America. Over 500,000 cases were reported, with fatality rates as high as 35%. The organism responsible was resistant to chloramphenicol, tetracycline, streptomycin, and sulfadiazine, all drugs commonly used to treat dysentery (Elwell and Shipley, 1980). It is not uncommon for bacterial plasmids to carry resistance traits for 6 or 7 antibiotics, with each trait determined by a single gene.

Similarly, development of resistance to insecticides can be of major economic, medical, and ecological importance. Georghiou and Taylor (1976) reported that at least 57 commercial pesticides were at that time affected by resistance, including DDT, most cyclodienes, and many organophosphates and carbamates. In addition, this number represented a 62.5% increase over the number of control agents affected in 1967, which suggests that current data would probably show greatly increased figures. Cross-resistance and multiple resistance also occur. The ramifications of resistance include financial losses to pesticide developers, marketers, and users; medical impacts from the inability to control disease vectors; and ecological disruptions due to severe imbalances in insect populations. Resistance usually involves subtle changes in insect biochemistry or physiology resulting from mutations followed by intense selection. The most common mechanisms of resistance at the biochemical level involve 1) alterations of a target enzyme that decrease its sensitivity to a pesticide; 2) decreased absorption; or 3)detoxification. Although the mechanisms of resistance are diverse, they have usually been shown to be determined by single gene changes. In the case of DDT resistance, three different mechanisms are known, each of which, in concert, produces resistance intensities that are multiplicative rather than additive.

The role of single gene mutations can also be dramatic in producing shifts in host/parasite relationships, particularly in increasing host range or in overcoming host resistance. Simberloff (1985) cites the example of the apple maggot, Rhagoletis pomonella. This fly pest was almost wholely restricted to hawthorn until the mid-19th century when it suddenly began to attack apples in the northeastern United States. The apple race subsequently spread throughout much of the country. Sufficiently good records of the host range shift and genetic analysis of enough of the fly's traits are available to provide convincing support to the hypothesis that the host range shift resulted from two mutations. Nester et al. (1985) recently reported that resistance to crown gall disease in certain grape vine strains is determined by a single gene in the pathogen, Agrobacterium tumefaciens, which is recognized by the host plant. Without this gene, the plant's resistance mechanism breaks down, so that even deletions of single genes can be important in host range shifts. Finally, Pimentel (1985) also provides numerous examples of single gene changes that allowed both insect and microorganism parasites or pathogens to overcome host resistance mechanisms. Notable examples include agricultural rusts and the Hessian fly.

Unfortunately, however, a genetic basis has not been identified specifically for success in invasion events. Ehrlich (1986) states that "we cannot list in detail genetic attributes present in some species but absent in close relatives, that have provided advantages...in novel environments." Nevertheless, ecologists and evolutionary biologists believe that it is not the number of genes that are modified but rather which genes they are that determines the need for evaluating whether large or important ecologically

relevant effects will result. From the ecological viewpoint, the number of genes added to or subtracted from an organism's genome is not the standard against which to judge the necessity for risk analysis, and the differing magnitudes of "new" genetic information in engineered vs exotic organisms does not negate the validity of the introduced species model.

Convergent Evolution: Genotype vs Phenotype

When organisms that are not closely related evolve similar characteristics because of independent adaptation to similar environmental situations, they are said to have undergone "convergent" evolution. Ernst Mayr (1970) said, "The animal world is full of convergences (and so is the plant world!) where similar demands by the environment have evoked similar phenotypic responses in unrelated or at least not closely related organisms. If there is only one efficient solution for a certain functional demand, very different gene complexes will come up with the same solution, no matter how different the pathway by which it is achieved." Well known examples of convergence include similar adaptations to a marine existence in fish and whales; the jointed legs and hinged jaws of arthropods and vertebrates; and the remarkable similarities between marsupial and placental forms which frequently play the same ecological roles despite extreme evolutionary and geographic separations.

Convergent evolution illustrates the point that knowledge of the genotype of an organism does not necessarily allow a prediction of phenotypic expression in an ecological context. In fact, there may be a considerable disconnect in making inferences from one to the other. Very different genotypes may produce startlingly similar ecological role players. Very similar genotypes may, on the other hand, produce unexpected ecological differences. Again, it is not the number of similarities or differences at the genetic level that is important, but rather where those similarities or differences reside and how they perform ecologically that is important.

Similarities of Process

Finally, the introduced species model represents a valid framework for evaluating the ecological effects of genetically engineered organisms because the processes by which an added organism penetrates an ecological community, commandeers resources, and possibly exerts effects on other organisms are the same regardless of the origin of the element, or elements, that make that organism "foreign," "new," or "different." The ecological events that determine success or failure are the same whether the organism is moved from one continent to another, from one ecosystem to another on the same continent, or from a laboratory to an open field. Many of the same questions apply, such as "What characteristics does this organism have that might allow it to persist in this particular environmental context?" and "What will be the consequences to other species and to the ecological processes in which they participate if it does persist?"." The kinds of modifications that are of interest to biotechnology (e.g., plants able to grow at lower temperatures or nutrient levels, microorganisms with expanded ranges of nutrient sources or host species) are hardly minor changes from an ecological viewpoint. The novel traits of an engineered organism may give it superior new ability to survive in a different habitat or on a new food source. If this is so, then that organism is very much the ecological equivalent of an introduced species in an environment where it did not exist before.

What Can We Do With the Lessons?

There is currently disagreement among ecologists as to the nature of the causes that produce the kinds of patterns discussed earlier. The leading spokesman for the school that insists upon rigorous hypothesis testing in drawing inferences about such patterns is Dan Simberloff. Simberloff is skeptical of the validity of the generalizations made above because he maintains that few data sets are available to rigorously test the hypothesis that "simple" or "undersaturated" communities are more vulnerable than "species rich" systems. He furthermore maintains that compiling lists of general species traits and tabulating numbers of species present or absent is "interesting" but inadequate to allow satisfactory prediction of whether a particular introduction will succeed or not.

Simberloff's quarrels are primarily, however, over the methodology used to draw inferences and should not be mistaken as disagreement that introductions of new organisms can have serious effects. Understanding him in this mistaken way would be rather like taking the fact that some evolutionary biologists disagree with the concept of gradualism as the predominant evolutionary mechanism to mean that these individuals do not believe that evolution occurs. In one paper (Simberloff, 1981), for example, Simberloff presented an evaluation of published literature on introduced species in which he interpreted the data to indicate that the majority of introductions apparently do not result in negative ecological effects. This paper has strongly lent itself to misinterpretation. First, Simberloff admitted in this paper that the data he had to work with may have been biased by a lack of documentation of damage. Second, the fact that he emphasized that 678 of 854 introductions appeared to have had no negative effects should not be taken to mean that he dismissed the importance of the negative effects, including 71 extinctions, that did result from the other 176 cases. His interpretation that the data suggest that most introductions do not cause negative effects is not really a source of disagreement with other ecologists. Sharples (1982), for example, stated that "the number of organisms that have become established is only a fraction [of the thousands that have been spread from their native homes]...and the number that cause ecological disruptions is a still smaller fraction."

Finally, whether Simberloff's accounting system for negative effects is right or wrong misses the point. The most discomforting aspect of introducing novel organisms into the environment, whether "exotic" or genetically engineered, is not that some of them may be harmful, but rather that it will be difficult to know which ones are. Although his skepticism of the generalizations is contradictory to the views of many other ecologists who have made a great many empirical observations that fit them, in one regard Simberloff is undoubtedly correct. Such generalizations are not sufficiently detailed to produce credible risk assessments for engineered organisms. In no way, however, has Simberloff suggested that risk assessment is unnecessary. In fact, in his 1985 paper, he was addressing the necessity for risk assessment when he stated, "That there are no simple predictive laws based on a few parameters does not mean that ecologists are powerless to understand the effects of introductions...[I]t takes very detailed empirical study in most cases to understand how one species or genotype affects others. Such understanding is certainly possible...but often requires field research of about the depth of a doctoral dissertation." He also provided a few hopeful

words. "Intensive, species-focused field and laboratory research has allowed post facto explanation and occasionally prediction on a case-by-case basis, and there is every reason to think that greater effort of this sort will usually succeed."

What then is the value of the lessons from introductions of exotic organisms? First and foremost, our knowledge of the dynamics and effects of introduced species strongly suggests that there is real potential for negative ecological effects to result from the release of at least some genetically engineered organisms. This potential should be taken seriously and justifies the use of risk assessment. The goals of risk assessment will be to obtain accurate prerelease knowledge of the behavior an organism is likely to exhibit in its new ecological context and given its particular genetic modification; to be able to detect and avert potential problems before they occur; and, in general, to exert greater control over the outcome of the introduction than has been characteristic of most past experience.

Second, the vast amount of documentation available on introductions of exotic species does contain valuable information. Much of this information has not yet been deeply probed, and it is still likely that the general principles we have currently could be considerably refined with more comprehensive and in-depth analyses. If nothing else, such general principles may provide guidance for distinguishing cases that have the potential to be particularly problematic, e.g. release of broad host range species in highly undersaturated communities.

For adequate risk assessment now and in the future, however, research that is directed at the specifics of the organism to be released and the environment that will receive it is essential. Organisms about which a great deal is already known (e.g., many crop plants) will require less effort than those for which the information base is scant (e.g., most microorganisms). The level of detail that will be required in risk assessment for genetically engineered organisms will have to be determined by a consideration of the characteristics of the unmodified parental organism, including its ecological role; the nature and effects of a particular genetic modification on the organism's ecologically significant phenotypic traits; and the specific environment in which it will be used.

Literature Cited

1. Alexander, M., "Ecological Consequences: Reducing the Uncertainties," Issues in Science and Technology 1(3):57-68, 1985.
2. Crawley, M.J., "What Makes a Community Invasible?" presentation at British Ecological Society Symposium, Colonization, Succession, and Stability (Oxford, England: Blackwell Scientific Publications, 1986).
3. Diamond, J. and Case, T.J., "Overview: Introductions, Extinctions, Exterminations, and Invasions," Community Ecology, J. Diamond and T.J. Case, (eds.) (New York: Harper & Row, 1986).

4. Dritschilo, W., et al. "Implications of Data on Introduced Species in California for Field Releases of Recombinant DNA Organisms," Environmental Sciences and Engineering Report No. 85-60, (Los Angeles: University of California, April 25, 1985).

5. Ebenhard, T., "Introduced Birds and Mammals and Their Ecological Effects," Swedish Wildlife, in press.
6. Ehrlich, P.R., "Which Animal Will Invade?," Ecology of Biological Invasions of North America and Hawaii, H.A. Mooney and J.A. Drake, (eds.) (New York: Springer-Verlag, 1986).
7. Elton, C.S., The Ecology of Invasions By Animals and Plants, (London: Chapman and Hall, 1958).
8. Elwell, L.P. and Shipley, P.L., "Plasmid-mediated Factors Associated with Virulence of Bacteria to Animals" Ann. Rev. Microbiol. 34:465-496, 1980.
9. Georghiou, G.P. and Taylor, C.E., "Pesticide Resistance as an Evolutionary Phenomenon" Proc. of XV International Congress of Entomology, (College Park, MD: Ent. Soc. of America, 1976).
10. Mayr, E., Populations, Species, and Evolution (Cambridge, MA: Bellknap Press, 1970).
11. Moyle, P.B., "Fish Introductions into North America: Patterns and Ecological Impact," Ecology of Biological Invasions of North America and Hawaii, H.A. Mooney and J.A. Drake, (eds.) (New York: Springer-Verlag, 1986).
12. Nester, E.W., Yanofsky, M.F., and Gordon, M.P., "Molecular Analysis of Host Range of Agrobacterium tumefaciens," Engineered Organisms in the Environment: Scientific Issues, H.O. Halvorson, D. Pramer, and M. Rogul, (eds.) (Washington, DC: American Society for Microbiology, 1985).
13. Pimentel, D., "Using Genetic Engineering for Biological Control: Reducing Ecological Risks," Engineered Organisms in the Environment: Scientific Issues, H.O. Halvorson, D. Pramer, and M. Rogul, (eds.) (Washington, DC: American Society for Microbiology, 1985).
14. Pimm, S.L., "The Complexity and Stability of Ecosystems," Nature 307:321-6, 1984.
15. Pimm, S.L., "Community Stability and Structure," Conservation Biology. The Science of Scarcity and Diversity, M.E. Soule, (ed.) (Sunderland, MA: Sinauer Associates, Inc., 1986).
16. Post, W.M. and Pimm, S.L., "Community Assembly and Food Web Stability," Math Biosci 64:169-92, 1983.
17. Regal, P.J., "Models of Genetically-Engineered Organisms and Their Impact," Ecology of Biological Invasions of North America and Hawaii, H.A. Mooney and J.A. Drake, (eds.)(New York: Springer-Verlag, 1986).
18. Sharples, F.E., "Spread of Organisms with Novel Genotypes: Thoughts from an Ecological Perspective," ORNL/TM-8473, (Oak Ridge, TN: Oak Ridge Natl. Lab., 1982). (Also Recombinant DNA Technical Bulletin 6(2):43-56, 1983).
19. Simberloff, D., "Community Effects of Introduced Species," Biotic Crises in Ecological and Evolutionary Time, M.H. Nitecki, (ed.) (New York: Academic Press, 1981).
20. Simberloff, D., "Predicting Ecological Effects of Novel Entities: Evidence From Higher Organisms," Engineered Organisms in the Environment: Scientific Issues, H.O. Halvorson, D. Pramer, and M. Rogul, (eds.)(Washington, DC: American Society for Microbiology, 1985).
21. Simberloff, D., "Introduced Insects: A Biogeographic and Systematic Perspective," Ecology of Biological Invasions of North America and Hawaii, (New York: Springer-Verlag, 1986).
22. Vitousek, P.M., "Plant and Animal Invasions: Can They Alter Ecosystem Processes?" Engineered Organisms in the Environment: Scientific Issues, H.O. Halvorson, D. Pramer, and M. Rogul, (eds.) (Washington, DC: American Society for Microbiology, 1985).

IMPACT ON COMPETITIVE ABILITIES OF SPECIFIC GENETIC ALTERATIONS

Stuart L. Pimm
Department of Zoology
University of Tennesee

and

Bruce R. Levin
Department of Zoology
University of Massachusetts

While evolutionary biologists may disagree about the relative importance of the different processes leading to changes in the genetic structure of populations (evolution), all would agree that natural selection operating on genetic alterations, mutations in the broad sense, is the dominant force responsible for the adaptation of organisms to their environment. In the classical view, the incidence and nature of the genetic alterations that serve as the raw material for adaptation are independent of the selective pressures responsible for their ascent (Luria and Delbruck, 1943). Stated another way, adaptation is the end product of random alterations in the genotypes of individuals.

In addition to genetic alterations that occur within populations, genetic alterations can also be acquired by outcrossing and recombination. As a result, the genetic changes that lead to adaptation in one population may have originated in another population, where they have already passed through the gauntlet of natural selection. In the eukaryotes, outcrossing and recombination is (or at least thought to be) limited members of the same or very closely related species. In bacteria, genetic exchange is an infectious process in which accessory elements (plasmids, phage, transposons) and possibly chromosomal genes are transferred (directly or indirectly) among individual cells of the same and different species. In bacteria, this external source of genetic alteration plays an important role in adaptation. The rapid rise of antibiotic resistance during the past 30 years is an excellent (and frightening) example of this process (Falkow, 1975).

Since adaptation can be a consequence of selection for random genetic alterations, it is not possible to say with certainty that specific genetic alterations (be their origins synthetic or natural) would not increase the ability of an organism to survive, reproduce, compete for resources or invade new habitats. Stated another way, there is no justification for a general assertion that genetic alterations will always lower the fitness of an organism. On the other hand, there could be justification for less general and more quantitative statements about the ecological consequences of genetic alterations. (1) Are there classes of genetic alterations that are likely to have a negative effect on the fitness of an organism? (2) Is it possible to ascertain the probability that a specific, well characterized, genetic change would be neutral or augment fitness? (3) Is the effect of a genetic alteration on fitness likely to be conditional, to depend on environmental or other factors? (4) Are genetic alterations acquired by recombination from the same or closely related species more likely to augment fitness than those acquired from distantly related species?

In the first portion of this essay we examine these four questions and how well they can be answered in general and in specific, case-by-case, situations. We then consider the impact that introductions of genetically modified and impaired organisms would have on natural communities.

Classes of Genetic Alterations

The phrase "genetic alteration" includes a large number of processes by which the genomes of organisms are modified naturally and by human intervention. To facilitate this discussion, it is useful to group these genetic alterations into a modest number of classes.

1- Simple mutations: small modification in which the general organization of the genome is not altered. Included among these are changes in nucleotide composition; base substitutions, deletions and additions of relatively few bases. The incidence of simple mutations can be augmented by the use of chemical mutagens or ionizing radiation. While in the past it was not possible to control the nature of simple mutations, there are now procedures for "site specific mutagenesis", where particular sequences of bases are inserted into predetermined genes (Traboni et al., 1983). Simple mutations can alter the phenotype of an organism by changing the quantity or nature of the products of structural genes or the signals that control the action of these genes. These phenotypic changes can involve single characters or multiple characters (pleiotropy).

2- Reorganizations: large-scale rearrangements of the organization of the genome. Included in this class are changes in chromosome number (polyploidy and polysomy), changes in the positions of chromosomal regions (transposition), changes in the order of chromosomal regions (inversions), and multiplications, deletions and additions of chromosomal regions. The incidence of reorganizations may be augmented by mutagenic chemicals and ionizing radiation and, as in the classic case of Zea maize (McClintock, 1951), these reorganizations can result from the movement of transposable elements. At this juncture, it is not possible to artificially induce specific, large-scale rearrangements of the genome. Reorganizations can affect the phenotype of an individual in the same way as simple mutations, but their effects are likely to be more profound and more encompassing. In sexually reproducing organisms, reorganizations can also lead to hybrid sterility.

3- Accessory elements: the insertion or excision of transposons and proviruses into chromosomes or established accessory elements and the addition or deletion of plasmids and proviruses. Accessory elements can affect the phenotype of an organism by carrying genes that code for host-expressed phenotypes, by modifying the expression of existing genes, or by causing genomic rearrangements.

Now to address the questions posed.

Are There Classes of Genetic Alterations that are Likely to Have a Negative Effect on the Fitness of Organisms?

Accepting the largely theoretical concept of the genome being a coadaptive complex the component parts of which evolved with respect to each other (e.g. Lerner, 1954), this question has to be answered in the affirmative. Save

possibly for simple mutations that do not change the activity, timing, or properties of a gene product, one would anticipate genetic alterations would generally be at least slightly deleterious. A change in one region of the genome (simple mutation), a change in the relative dosage of genes (reorganizations) or the addition or deletion of an accessory element would interrupt the balance of the coadapted complex.

Although the empirical evidence in support of this coadapted gene complex interpretation is less than overwhelming, there is some natural population evidence in support of it, see for example the studies of the genetic structure of intertidal populations of copepods Burton (1986). On the other side, there is evidence, that simple mutations that alter products of structural genes may not engender much of a cost, unless they preclude the production of a functional product. In a study of mutations of the LacZ (B-galactosidase) gene of Escherichia coli, Langdrige and Campbell (1969) found that only 5% of the lacZ- base substitution mutations were missense (coded for a wrong amino acid). Since they expected a ratio of missense to nonsense (chain-terminating) mutations of 94:6, they concluded that "most amino acid replacements in B-galacosidase do not decrease enzyme activity sufficiently to prevent growth on lactose". While not evidence that these mutations do not effect fitness--there is no way of making inferences of this type--these results do suggest that structural genes are robust and simple mutations in them may not be perceived by natural selection, even when that gene product is needed. Consistent with this interpretation is the existence of large numbers of different, functional, B-galactosidase alleles in natural populations of E. coli (Selander and Levin, 1980).

It might seem that as a class, simple mutations that eliminate the function of a normally active gene would necessarily engender a cost in fitness. This is certainly be the case when the affected gene is needed for growth and survival, for example the genes needed for the synthesis of tryptophane in an environment where this amino acid is not present. But here too, a general conclusion is not possible. These "negative" mutations could be favored in environments where that gene is not needed (Zamehof and Zamehof, 1969; Dykhuizen, 1978).

Evidence from humans certainly suggests that major genomic reorganizations would be selected against. Individuals with Down's syndrome, Klinefelters syndrome and other chromosomal abnormalities would, under most natural conditions, be at a disadvantage. Indeed, karyotype studies of spontaneously aborted fetuses suggest that the majority of major chromosomal mutations are incompatible with life (Carr and Gedeon,1977). On the other hand, it is clear that a significant amount of evolution is reflected in changes in number organization of chromosomes (White, 1977). While we can't say with certainty that these reorganizations served as the raw material for that evolution, it would seem that they were unlikely to have been very deleterious.

In the absence of direct selection for genes borne by plasmids, proviruses and transposons, one might anticipate that as a consequence of the burden (in added DNA and protein synthesis) imposed by their carriage, these elements would be deleterious their host. There is, in fact evidence that unselected plasmids reduce the fitness of their hosts (Levin, 1980; Freter et al., 1983). The P-elements (transposons) in Drosphilia certainly appear to be deleterious to flies that had previously not carried them (Kidwell, 1986). On the other

side, there is also evidence that even without direct selection for specific genes, prophage and transposons augment the fitness of their host bacteria (Edlin et al., 1977; Dykhuizen et al. 1978; Biel and Hartl, 1983). At this time, the mechanisms responsible for these accessory-element determined enhancements of host fitness have not been elucidated. However, there is some evidence that transposons can augment host fitness by simply by acting as mutators (Chao et al., 1983).

Taking the theoretical ideas about coadapted gene complexes together with the limited empirical evidence available, it seems that the best one could offer is an equivocal answer to the question of the existence of classes of genetic alterations that would be negative, viola. While one could make a reasonable case that the majority of genetic alterations with phenotypic effects would be deleterious, there is no single class of genetic alterations that would necessarily be deleterious.

Is it Possible to Estimate the Probability that a Specific Genetic Alteration Would Have Neutral or Positive Effects?

In the case of some well-characterized genetic alterations, it may well be possible to obtain rough estimates of the likelihood of their being neutral or under positive selection. However, it would seem that these estimates can only be obtained for a modest number of genetic alterations, and one would be able to put little confidence in their accuracy.

Based on that which we know about transcription, translation and gene action, it would seem that structural gene mutations leading to synonymous codon changes are very likely to be selectively neutral (Kimura, 1983). As indicated above, this may also be the case for some structural gene mutations that affect the primary or higher-order structure of the protein. However, ascertaining the likelihood of a mutation of the latter type being neutral would require more information about that gene's product, pleiotropic effects and the ecological role of the phenotype code for that gene than is generally available for most genes and organisms.

If one had extensive information about the environment into which an organism was going to be released and about the phenoytpe coded for by the genetic alteration, then it would be possible to predict whether that modification would be favored by selection. For example, if an antibiotic resistance plasmid were introduced into a clone of antibiotic sensitive bacteria whose natural habitats included the guts of humans or domestic animals, one could say with some assurance that genetic alteration would be favored in at least some situations encountered by that clone. It is, in fact, possible that the carriage of an antibiotic resistance plasmid, would allow bacterial clones to colonize habitats that they could not otherwise invade.

The question is, how often would one have the necessary environmental and phenotypic information to assess the likelihood that a particular genetic alteration would be favored? It seems that in general this information would not be available. On the other hand, this information probably could be obtained in many (but not all) situations where the need for this information is critical. Stated another way, on a case-by-case basis it might be possible to ascertain whether a specific genetic alteration would have positive effects.

Is the Effect on Fitness of a Genetic Alteration Likely to be Conditional?

This question raises the major caveat to making predictions about the fate of genetic alterations. One may conceive of genetic alterations with unconditional phenotypic effects, such as lethals. However, save for the ecologically uninteresting case of absolute lethals, it seems reasonable to assume that the phenoytpes determined by any genetic alteration would, to some extent, be influenced by either the genetic background of the organism or the environments in which it lives. Thus, at a qualitative level, this question has to be answered in the affirmative. But there is more to it than that.

It is easy to add empirical credence to the assertion that one can anticipate significant environmental and genetic background effects on the phenotype determined by specific genetic alterations. When bacterial populations are confronted with virulent bacteriophage, phage resistant mutants rapidly ascend and become the dominant cell types (Chao et. al, 1977). However, in the absence of phage, these resistant mutants are almost invariably at a disadvantage (Lenski and Levin, 1985). Even the selective neutrality of structural gene variants can be conditional. Under some conditions, selection could distinguish among seeming neutral allozymic variants of 6-phosphogluconate dehydrogenase, Hartl and Dykhuizen (1981)

In the earlier, less molecular, days of genetics, there were two terms in common use, "penetrance" and "expressivity". King and Stansfield (1985) define penetrance "as the proportion of individuals of a specified genotype that show the expected phenotype under a defined set of environmental conditions"; and they define expressivity as "the range of phenotypes expressed by a given genotype under any given set of environmental conditions or over a range of environmental conditions". As an example of expressivity, King and Stansfield consider the observation that Drosophila that are homozygous for the recessive gene "eyeless" may have phenotypes varying from no eyes to completely normal eyes. In the laboratory, geneticists tend to work with "good markers", genes with complete penetrance and expressivity. This, however, reflects a considerable bias. They choose the genes to work on because they are "clean", and most commonly study those genes in organisms with little (or no) variation in genetic background and little environmental effects. Even then, phrases like "leaky" often creep into their descriptions of those genes.

Having now convinced you that one should anticipate the effects on fitness by specific genetic alterations to be conditional, it necessary to ask whether it matters. From an academic perspective, the conditional nature of fitness is certainly of interest. Save for idealized (an unanticipated) situations where a genetic alteration is in complete linkage equilibrium and maintains that state, genetic background affects on fitness would certainly influence the rate and dynamics of the ascent or exclusion of a genetic alteration. Environmental effects on the fitness would almost invariably affect the rate and dynamics of a genetic alterations' passage through a population and whether it will be maintained in a polymorphic or monomorphic state, or persist at all. From the present, more practical perspective, the fact that one has to assume that the effedts of specific genetic alterations are almost invariably going to depend on genetic bakground and environmental conditions--in ways that are likely not to be known--certainly limits the ability to make predictions about the fate and ecological consequences of these alterations.

Are Genetic Alterations Acquired by Recombination with the Same or Closely Related Species More Likely to Augment Fitness than Those Acquired From Distantly Related Species?

In the not-to-distant past this question would not have been of very much interest. In sexually reproducing eukaroytes, the biological species concept precludes gene exchanges among organisms of different species: if organisms normally exchanged genes they would be defined as members of the same "species". While for organisms that reproduce asexually, the concept of species is not well developed (firm), nevertheless one would still assume that the gene exchanges that did occur would be primarily among members of the same, taxonomically defined, species. Now, and for the past 12 years, this question of interspecific gene exchange is of considerable interest. By human intervention, in vitro genetic engineering, it is possible to produce functional genetic alterations by the insertion of genes from one species into another, very different species. The recent example of firefly lucerferase genes in tobacco (Ow et al., 1986) is certainly a dramatic illustration of this. Indeed, if not for the recent expansion of the possibilities for genetic alteration by interspecific gene exchange, we would probably not be engaged in the present exercise.

If one accepts the concept of the genome being a coadapted complex, one would tend to answer this question in the affirmative. Genes acquired from members of the same closely related species are more likely to fit in with the rest of the genome than those from organisms with a very different genetic background. If those acquired genes coded for phenotypes that were favored by selection, and were from an organism with a similar genetic background, they are more likely to have a net fitness advantage than if their presence upset the balance of the coadapted complex.

As is not uncommonly the case in this debate, one can also make a case for the alternative side of this issue. Interspecific gene exchanges represent a way to overcome the limitations of evolutionary constraints. As a consequence of prior evolution, the existing structure of an organism's genome may preclude the production of genetic alterations that would enable it to generate particular phenotypes, which would adaptive. This constraint would also be present in closely related species. On the other hand, there could well be a distantly related or even unrelated species in which the necessary genetic alteration has already occurred and become established. The advantage accrued by acquisition of that genetic alteration from another species may well exceed the costs engendered by upsetting the existing coadapted gene complex.

The ease with which one can produce plausible arguments on both side of this issue and dearth of data addressing this question of the interspecific gene exchange, lead to a single answer. <u>Thereis no justification for assuming that, categoricay, recombinants generated by exchanges between members of the same or closely related species are more or less likely to be favored by selection than those produced by gene exchanges among members of distantly related species.</u>

Impact of Genetic Alterations on Competitive Abilities

In this section, we consider whether a genetically-modified organism, particularly one that might be considered genetically handicapped, may be capable of having an impact on the community of organisms into which it is

released. There are two parts to this section. In the first, we consider the ideas relating to how species evolve. Organisms might be expected to evolve towards increasing fitness, but genetic changes, particularly large ones, often significantly reduce fitness. Are there circumstances under which organisms with genetic handicaps can spread? In the second part, we shall show that some species genetically modified by man, by conventional breeding programs, are not only capable of surviving when introduced to the wild, they are capable of thriving. Indeed, some of the most damaging species introductions have been of genetically-modified species.

The Potential for Spread Among Genetically-Handicapped Organisms

Species are often envisioned, metaphorically, as sitting on "peaks" of adaptation. The adaptive-peak metaphor raises a problem about the process of speciation. By definition, peaks are surrounded by valleys and natural selection pushes populations up peaks, towards increasing adaptation. Evolving from one peak to another requires an initial period in which the population moves down. Many adaptations are polygenic, and complex adaptations are readily disrupted by even single mutations. So how can organisms with complex, polygenic adaptations change significantly? Is it possible for organisms handicapped by man-made, genetic changes to survive and flourish, spreading the genes (and the handicap) through the population?

Two general solutions have been offered for the problem of evolving from one adaptive peak to another. The first centers around Wright's (1980) "shifting balance theory." Genetic drift can cause a population to move into an adaptive valley in opposition to natural selection. If the population moves far enough down to encounter the slope of another peak, then natural selection can take over. Genetic drift is a significant force only for small populations. This is one reason why small, isolated populations at the edge of a species range figure prominently in many discussions of evolutionary change.

The second solution invokes frequency- and density-dependent selection (Rosenzweig 1978, Pimm 1979, Wilson and Turelli, 1986). Imagine two niches, one of which is "filled" by a species and the other of which is "empty." A mutant type appears that is poorly equipped to occupy either niche, because it is handicapped by its genetic modifications. The mutant, however, is able to exploit the empty niche. Despite its handicap, the mutant form may still have a higher fitness than the wild type because its niche is "empty" while the old niche is "full." For example, inefficiently exploiting an abundant resource may be more profitable than efficiently gathering a depleted resource. In this fashion, the handicapped mutation can increase its frequency and persist long enough to be selected for increased efficiency. Note that this solution requires that there be empty niches.

Returning to the adaptive-landscape metaphor, peaks should perhaps not be envisioned as stable and unchanging like mountain peaks, but constantly shifting--their rise and fall governed by rules of density and frequency dependence. The fitness of a genotype, which determines whether it is on a peak or in a valley, is determined both by its own properties, such as its ability to exploit a resource, and by the properties of the environment, such as the characteristics and abundance of the resource. An efficient consumer of a depleted resource may then be in a valley compared to the inefficient consumer of an abundant resource, which is on a peak. Only after the inefficient consumer evolves to be more efficient and depletes its own

resources will the fitness surface resemble two peaks separated by a valley. In this fashion, empty niches can be colonized without invoking genetic drift. We see the possibility for genetically-handicapped organisms persisting and even flourishing, and thus, lasting long enough to be subsequently improved via natural selection.

These ideas have been developed in a number of ways. Verbal models, like the one we have presented, are powerful tools. But there is always the nagging suspicion that some essential condition may have been omitted. Mathematical models check on this consistency and give an indication of how easily a model may work. Early models (e.g. Pimm, 1979) used simple, differential-equation systems and assigned to different genotypes their growth rates, mortalities, and competitive abilities. Wilson and Turelli (1986) used an approach that derives these parameters from an explicit treatment of resource use, competition, renewal. Their conclusions are very similar to those of the early models.

Wilson and Turelli found that unexploited niches may be readily invaded by genetically-handicapped organisms. The handicapped organisms often may persist after organisms more efficient at exploiting the new resource evolve, thus paving the way for disruptive selection, in some circumstances.

Such theoretical ideas suggest an obvious question: is there evidence that species genetically modified by humans can spread, and if so, do they spread into niches that might previously been considered "empty?" To answer this, we turn to an examination of the literature on species invasions, particularly of vertebrates. Such a literature demonstrates that genetically-modified species can spread successfully and persist long enough to be the subjects of natural selection. Moreover, these species can have dramatic effects on the environment. Indeed, we shall show that some of the most serious impacts on environments have come from genetically-modified species.

The Impact of Genetically-Modified Organisms

Mankind has moved species around the world for millennia, sometimes by accident, but often deliberately. Some, like English garden birds a world away in New Zealand, are merely quaint curiosities. But other introductions have devastated entire floras and faunas. In the first part of this section, we ask whether successful species introductions are likely to have a serious impact. If they do, where should the impacts be most serious? In the second part of this section, we consider whether species genetically modified by man are capable of causing severe impacts. Most of the species introduced by humans are not of genetically modified species. But we can certainly reject the idea that genetically-handicapped organisms are of no threat to the environment. Some of the most damaging species introductions are of species genetically modified by man through conventional, breeding programs. Of course, these modifications are not necessarily the same as those that we can now engineer. We must stress, that what the studies of introduced have to tell us are not predictions of the effects of genetically-engineered organisms. Rather, the studies raise possibilities about the effects that these organisms may have.

The Impact of Introduced Species

A. Do introduced species have an impact?

The impact of introduced organisms raises two related questions: which organisms are more damaging when released and which ecosystems are most vulnerable? The existing data to answer these questions are many, scattered, and of very uneven quality. Interpreting these data is often difficult. One of the earliest summaries, Simberloff (1981) has come under intense scrutiny by Herbold and Moyle (1986) and Ebenhard (1987). A summary of the data in these studies is provided by table 1.

Simberloff reviewed 10 papers covering 850 animal and plant introductions. The basic results were:

(1) Less than 10% of the introductions caused species extinctions and there were only 71 extinctions. Of the 10 studies, Greenway (1967) reported the highest percentage of extinctions--that is, 30%, or 55 of the 71 extinctions overall. Greenway's book was devoted to avian extinctions. Simberloff argued that introductions add species to communities rather than causing replacements or destructions.

(2) Of Greenway's 55 extinctions, over 90% were on islands.

(3) Both overall, and within Greenway's study, predation was the principal cause of extinction--that is, 51 of the 71 extinctions overall.

Simberloff's study has been interpreted to mean that species introductions rarely have much of an impact. Herbold and Moyle (1986) show that this is not a reasonable conclusion. Examination of the three papers from which Simberloff tallied 525 of his 854 cases showed that his determination of 'no effect' generally conflicted with the conclusions of the original papers.

From Elton (1958), Simberloff extracted 241 instances of species introductions and reported that only 4 of them showed "any effect at all." But Elton's accounts include two species of rats, chestnut blight, spruce budworm and gypsy moths--introductions of considerable economic significance. Elton, however, concluded that only 4 species did not show an effect! Atkinson (1985), for example, provides a detailed discussion of the seriousness of the introduction of various rat species over the last few centuries.

Herbold and Moyle continue by pointing out that many apparently benign introductions are into highly man-modified habitats where much damage has already by caused by those modifications. Using California fishes as examples, they find that 48 of 137 species of freshwater fishes come from outside the state. Analysis of the habitats of these introduced species shows that 21 occur in such highly-modified habitats as reservoirs and farm ponds, another 21 occur mainly in moderately-modified habitats such as streams with altered flow and temperature regimes, and only 6 occur in near-pristine habitats. This last group include 4 species of trout and salmon introduced into fishless lakes, in which the fish altered invertebrate and amphibian populations. In all, 24 of 48 introductions have been documented as having a negative impact on the native fauna. The effects of the remaining 22 species are not known and only 2 appear benign. These latter two species are limited to artificial habitats, such as farm ponds and sewage treatment ponds.

Herbold and Moyle's conclusions are simple: species introductions usually have an effect. If they do not, it may be because we do not know whether there

is an effect or not. When we know there is not an effect, it is likely to be in habitats that are already so man-modified that damage may not be so noticeable.

B. Under which circumstances do introduced species have their most serious effects?

What should we expect the effect of an introduced species to be? Perversely, some useful insights can be obtained from studies of the effect of removing species from communities. This is perhaps not as strange as the idea might first appear. An introduction may be a success because its predators or competitors are few or absent in its new surroundings. Indeed, an absence of competitors may be a prerequisite for a genetically handicapped species to flourish. And, of course, the successful introduction may cause extinctions whose effects will cascade through the community. The vehicle for generating insights about introduced species are complex, differential equation models of food webs, (Pimm, 1982), but their main features are simple and obvious.

Removing a plant species from the base of a simple food chain destroys the entire system. But the loss of one of several plant species utilized by a generalized herbivore in a more complex system would have much less of an effect, because the herbivore is not so dependent on one species. These effects are obvious and well-known. Less obvious are the effects of removing species from the top of the food chains. Removing the predator of a monophagous herbivore probably leaves the plant at a lower density, but it is likely to survive. Special conditions are required for a predator to eliminate its sole prey, before it, too, becomes extinct. In a more complex system, the predator's absence may lead to a polyphagous herbivore exterminating all but the one resistant plant species which then regulates the herbivores' numbers.

There are, therefore, three "don'ts" for species introductions. Impacts will likely be severe when:

(1) Species are introduced into places where predators are absent. Examples would include the introduction of large herbivores to islands, or of predators that feed high in a food chain and lack predators as a consequence. In a similar view, we might expect introductions to have more severe impacts when competitors are absent.

(2) Polyphagous species are introduced.

(3) Species are introduced into relatively simple communities where the removal of a few plant species will cause the collapse of entire food chains.

Ebenhard provides dramatic evidence of the first effect, suggestive evidence for the second, and there are some compelling case histories to support the third.

By searching over 800 introductions, Ebenhard has identified those species that have an effect in changing abundance, species composition or in causing extinctions. Overall, we suspect that these effects on the native communities are likely to be missed (compare Simberloff's and Atkinson's comparisons of the effects of rats). Yet, the comparisons of the incomplete data are interesting. Herbivorous mammals introduced to continents were recorded as having effects on

the native community in 26% of 89 cases. Yet on oceanic islands, which lack predators, 50% of 363 introductions show effects on the native community.

Almost all of the herbivorous mammals have generalized diets, but among five introductions of specialized herbivores, none were recorded as having an effect on the native community. This is a statistically significant result, despite the frustratingly small sample size.

For the last feature, we can compare the fate of island and continental birds following the destruction of various plant species. In North America, there appear to be no extinctions of vertebrates attributable to the loss of chestnut trees following the introduction of chestnut blight. Chestnuts were locally one of the commonest trees. Contrast this with the situation in Hawaii discussed for, example, by Pimm, (1987). Some of the larger nectarivorous birds seem to have been differentially sensitive to extinction, when a few important nectar producing plants were exterminated by goats and pigs. Similarly, the threatened Palila, depends on the seed of one species of tree, the Mamane, which does not regenerate when large herbivores are present, and the Akiapolaau, and insectivore, depends on the presence of large Koa trees. Koa trees are endemic to Hawaii and are a popular source of wood for objects d'art intended for tourists.

Not surprisingly, the introductions of generalized herbivores to islands is often devastating--all three factors operate.

C. Which species have the most serious effects?

Ebenhard's review of the effects of species introductions provides a simple answer to this question: goats, cats, and rabbits. These species are noteworthy in being generalized herbivores and predators and their effects are most pronounced on simple, island systems where competitors and predators are absent. But these species are also species which have been genetically modified by man using conventional breeding activities. The cats and goats that have caused so much damage have been bred for considerable periods of time. The rabbits introduced have often, though not always, been domestic varieties.

Summary: Implications for the Release of Genetically Engineered Organisms

Our conclusions are simple:

1. Organisms with probably severe genetic handicaps are capable of surviving and even spreading in the environment. The conditions required for this spread include the reduction of competition which may come from those very handicaps conferring the ability to exploit new resources.

2. Not only is the spread of genetically handicapped organisms possible, it may be a major evolutionary process.

3. Species introduced by man may have a major effect on natural communities.

4. These effects on natural communities are more severe in some predictable

circumstances. As suggested by (1) these include the absence of competitors and predators.

5. Of the species introduced by man that have caused significant ecological damage, some of the most important introductions are of species genetically modified by man by conventional breeding programs.

The implications of these results for the release of genetically engineered organisms are simple. Genetic modification may impose a severe competitive handicap on those organisms. For example, some varieties of plants are sterile. But if the modifications fall short of making the organisms sterile, the existence of a handicap, does not mean that the organisms will necessarily perish. Depending on the biotic environment the organisms encounter, the organisms may survive, flourish, and persist long enough to be subject to evolutionary forces that may improve their fitness.

Second, there is ample evidence that introductions of species are capable of causing significant damage to existing communities. Current estimates of what percentage of introduced species damage native communities are inadequate. Simberloff's estimate is clearly too low: many of the effects of introduced species are missed. Many introduced species are in highly modified habitats where their effects might not be recognized. Despite these problems with the data, there are some obvious patterns to the variation in the impacts of introduced species. There is no doubt that some naturally occurring systems are particularly vulnerable to species introductions. We have no reason to believe this is not a general phenomenon: vulnerable ecosystems appear to be a fact of life.

Third, the organisms that cause damage to natural ecosystems are often genetically modified. We doubt that the genetic modifications lead to these organisms being destructive. It is more likely that man introduces his domestic animals preferentially. None the less, these genetically modified species are quite capable of surviving in appropriate habitats. These introductions demonstrate the need to consider the characteristics of the ecosystem into which species are to be introduced. Simply assuming that genetically handicapped species will not cause damage, because of these handicaps, is not sufficient.

Fourth, we have not considered which kinds of genetic modifications have led to what ecological changes and how these changes lead to effects on native communities when the species are introduced. The information on the 'which', 'what', and 'how' of the previous sentence is available. There is much information about the effects of species introductions and, in particular, the effects of genetically modified species introductions. But this information needs to be summarized and analyzed, before any conclusions are drawn.

TABLE 1. Summary of the Effects of Introduced Species

(a) Simberloff (1981)

Type of introduction	Number of introductions	Number having effect	% Having effect
Animals and plants	850	71	8
(Greenway's study)	184	55	30

(b) Ebenhard (1987)

Mammals on continents	89	23	26
Mammals on islands	363	182	50

(c) Herbold and Moyle (1986)

California fish	48	24	50-96*

(Of the 48 introductions, there is insufficient information on 22 to know whether they have an effect or not. Only 2 species are known to be benign.)

Literature Cited

1. Atkinson, I.A.E., "The Spread of Commensal Species of Rattus to Oceanic Islands and Their Effects on Island Avifaunas," Conservation of Island Birds, P.J. Moors (ed.) I.C.B.P. Tech. Pub. 3: 35-81, 1985.
2. Burton, R.S., "Evolutionary Consequences of Restricted Gene Flow Among Natural Populations of the Copepod, Tigriopus californicus," Bull. Mar. Sci. 39: In press, 1986.
3. Biel, S.W., and Hartl, D.L., "Evolution of Transposons: Natural Selection for Tn5 in Escherichia coli K-12," Genetics 103:581-592, 1983.
4. Carr, D.H., and Gedeon, M., "Population Cytogentics of Human Abortuses," Population Cytogenetics: Studies in Humans, E. B. Hook and I.H. Porter (eds.) (New York: Academic Press, 1977).
5. Chao, L., Levin, B.R. and Stewart, F.M., "A Complex Community in a Simple Habitat: an Experimental Study with Bacteria and Phage," Ecology, 58:369-378, 1977.
6. Chao, L., Vargas, C., Spear B.B., and Cox. E.C., "Transposable Elements as Mutator Genes in Evolution," Nature 303:633-635, 1983.
7. Dykhuizen, D.E., "Selection for Tryptophane Auxotrophs of Escherichia coli in Glucose-limited Chemostats as a Test of the Energy Conservation Hypothesis of Evolution." Evolution 32:125-150, 1978.
8. Dykhuizen, D., Campbell, J. and Rolfe, B.G., "The Influence of Prophage on the Growth Rate of E. coli," Microbios 23:99-113, 1978.
9. Ebenhard, T., "Introduced Birds and Mammals and Their Ecological Effects," Swedish Wildlife, in press.
10. Edlin, G., Lin. L. and Bitner, R., "Reproductive Fitness of P1, P2, and Mu Lysogens of Escherichia coli," J. Virol. 64:560-564, 1977.
11. Elton, C.S., "The Ecology of Invasions by Animals and Plants"(London: Methuen, 1958).
12. Falkow, S. Infectious Multiple Drug Resistance, (London: Pion Press, 1975).
13. Freter, R., Freter, R.F. and Brickner, H. "Experimental and Mathematical Models of Escherichia coli Plasmid Transfer in Vivo and in Vitro." Infect. and Immun. 39:60-84, 1983.
14. Greenway, C., Jr., "Extinct and Vanishing Birds of the World (New York: Dover, 1967).
15. Hartl, D.L. and Dykhuizen, D.E., "Potential for Selection Among Nearly Neutral Allozymes of 6-phosphogluconate dehydrogenase in Escherichia coli," Proc. Nat. Acad. Sci. U.S.A. 78: 6344-6348, 1981.
16. Herbold, B. and Moyle, P.B., "Introduced Species and Vacant Niches," Amer. Natur. 128: 751-760, 1986.
17. Kimura, M., The Neutral Theory of Molecular Evolution, (Cambridge, England: Cambridge Univ. Press, 1983.
18. Kidwell, M.G.,"Molecular and Phenotypic Aspects of the Evolution of Hybrid Dysgenesis Systems," Evolutionary Processes and Theory, S. Karlin and E. Nevo (eds.) (1986).
19. King, R.C. and Stansfield W.D., A Dictionary of Genetics, (New York: Oxford Press, 1985).
20. Langridge, J. and Campbell, J.H., "Classification and Intragenic Position of Mutations in the B-galactosidase Gene of Escherichia coli," Mol. and Gen. Genetics, 103:339-247, 1969.
21. Lenski, R.E. and Levin, B.R., "Constraints on the Coevolution of Bacteria and Virulent Phage: a Model, Some Experiments and Predictions for Natural Communities," Amer. Natur. 125:585-602, 1985.
22. Lerner, M., Genetic Homeostasis, (New York: Wiley Press, 1954).

23. Levin, B.R., "Conditions for the Existence of R-plasmids in Bacterial Populations," Forth International Symposim on Antibiotic Resistance, S. Mitsuhashi, L. Rosival and V. Krcmery, (eds.) (Berlin: Aviceum Prague, Springer Verlag, 1980).
24. Luria, S. and Delbruck, "Mutations in Bacteria from Virus Sensitivity to Virus Resistance," Genetics, 28:491-511, 1943.
25. McClintock, B., "Chromosome Organization and Gene Expression," Cold Spring Harbor Symp. Quant. Biol. 16:13-47, 1951.
26. Ow, D.W., Wood, K.V., DeLuca, M., de Wet, J.R., Helinski, D.R. and Howell S.H., "Transient and Stable Expression of the Firefly Luciferase Gene in Plant Cells and Transgenic Plants," Science 234:856-859, 1986.
27. 6. Pimm, S.L., "Sympatric Speciation: a Simulation Model," Biol. J. Linn. Soc. 11: 131-139, 1979.
28. Pimm, S.L., "Food Webs," (London: Chapman and Hall, 1982)
29. Pimm, S.L., "The Impacts of Introduced Species," Trends Ecol. Evol. in press.
30. Rosenzweig, M.L., "Competitive Speciation," Biol. J. Linn. Soc. 10:275-289, 1978.
31. Selander, R.K. and Levin, B.R. "Genetic Diversity and Structure in Populations of Escherichia coli," Science 210:545-547, 1980.
32. Simberloff, D., "Community Effects of Introduced Species," Biotic Crises in Ecological and Evolutionary Time, M.H. Nitecki (ed.) (New York: Academic Press, 1981).
33. Traboni, C., Cortese, R., Cilibert, G. and Cesarini, G., "A General Method to Select M13. Clones Carrying Base Substitution Mutants Constructed in Vitro," Nucleic Acid Res. 11:4229-4239, 1983.
34. White, M.J.D. Animal Cytology and Evolution, Third Edition (Cambridge, England: Cambridge University Press, 1977).
35. Wilson, D.S. and Turelli, M., "Stable Underdominance and the Evolutionary Invasion of Empty Niches." Amer. Natur. 835-850, 1986.
36. Wright, S., "Genetic and Organismic Selection," Evol. 34:825-843, 1980.
37. Zamenhof, S. and Eichhorn, H., "Study of Microbial Evolution Through Loss of Biosynthetic Functions: Establishment of 'Defective' Mutants," Nature 216:455-458, 1967.

THE BIG AND SMALL OF IT: ARE THERE DIFFERENT RISKS TO RELEASING GENETICALLY ENGINEERED MACRO-ORGANISMS AND MICRO-ORGANISMS?

Kathleen H. Keeler
School of Biological Sciences
University of Nebraska--Lincoln

and

Andrew P. Dobson
Department of Biology
University of Rochester

The diversity of uses proposed for genetically engineered organisms to be released into the environment is truly staggering. (e.g., Teich et al. 1985, Vidaver 1985, Stern 1986). To evaluate the hazards of these diverse activities is a vast project unless unifying factors can be found. This paper considers whether there are risks common to both plants and animals ("macro-organisms") and bacteria, fungi and viruses ("micro-organisms").

The Concerns

Basic to all arguments about the risks of deliberate release of any non-native plant or animal are decisions about kinds of hazards that are tolerable or intolerable. Certainly something that causes substantial loss of human life is unacceptable. Furthermore, given our society's general intolerance of grizzly bears in the back yard, it is likely that things with minuscule but finite chances of killing humans will not be permitted, even if low general risk and large benefit can be documented. Likewise, organisms which produce substantial human illness, even if it never culminates in death, will not be tolerated.

These two items reflect one key difference between macro and microorganism deliberate release. No one is likely to release large animals which can kill humans. (Technically cows and pigs can kill humans, but we have lived comfortably with this hazard a long time and new breeds of livestock are unlikely to change it.) Potentially lethal plants are also tolerated, as the widespread planting of known dangers as castor bean (Ricinus communis) and oleander (Thevetia peruviana) indicate (Lewis and Elvin-Lewis 1977, Baldwin 1979). The same is NOT the case for potentially toxic micro- organisms. Here we are dealing with potential disease situations, and people feel very strongly against the release of pathogenic organisms and of encountering such organisms unexpectedly. Thus, the regulatory situation is much more important for potentially lethal micro-organism than macro- organisms, because of a much stronger public antipathy. The causes are unclear: perhaps it is simply easier to catch and kill an escaped tiger than an escaped Salmonella bacterium, but, in any event, people seem more willing to trust the existing regulatory structure to protect them from dangerous macro-organisms than from dangerous micro-organisms.

The second level of concern is that the released organisms injure animals and plants, particularly animals and plants of economic or emotional value (crops, livestock, pets). Release of organisms with a reasonable probability of causing economic losses in agriculture will be prevented (if foreseen); the

USDA APHIS and animal quarantine laws were enacted to prevent such problems and will continue to function to prevent economic losses to agriculturally important organisms.

Thirdly, introduced organisms may disrupt natural communities, causing increases or decreases in species abundance, possibly to the level of local or global extinction. Tolerance of released organisms which disrupt the environment quickly becomes an economic and political issue, based perhaps loosely on biological grounds. Non-engineered animals have been released in the past, either because people liked them (English sparrow in North America) or because people thought they would be economically useful (carp in North America) or solve a problem (mongooses to catch rats in Hawaii). Whether these are disasters or successes depends on (i) the value put on native communities with native species, (ii) the value put on having exotic animals in the ecosystem and (iii) the relative success of the animals. For example, feral cats and dogs do not raise widespread outcry in North America, probably because of low values on all three counts, despite the serious damage they can do as predators (see Pimm and Levin, this volume). Comparable stories exist for plants. While we have many undesirable alien plants, some successful aliens are not even generally recognized as nonnative, for example Eucalyptus in California.

For micro-organisms, their presence or absence is rarely noted except in their impact on macro-organisms. Thus the concern about release of novel micro-organism will chiefly be in regard to their impact as disease organisms. If they cause, like Dutch elm disease, loss of visible and economically important groups, their introduction was a serious error. If they slip into the ecosystem unnoticed, or occasionally do some good restricting pest species, as in the case of myxomatosis, then there would be little objection.

Finally, released organisms might have little visible impact but ultimately disrupt ecosystem processes, such as the movement of nitrogen. These scenarios more often concern the introduction of micro than macro- organisms, because in general micro-organisms fill the key roles in these cycles (Alexander and Andow 1986, Cairns and Platt 1986). Further amplification of potential problems from ecological disruption are considered under **Cascade Effects**.

One fundamental difference we see, then, between the regulation needs for macro-organisms and micro-organisms is that people will more often notice a new macroorganism and make value judgments about its desirability. Based on the number of people who will notice the new species, less regulation is required for micro-organisms.

However, people are generally more afraid of wild micro-organisms than wild macro-organisms. A news report of release, it seems to us, will provoke more public furor for a microorganism than a macro-organism, other things being equal (consider for example, your reaction to escape of "an experimental virus" vs "an experimental plant"). The best we can document this impression is to look at the field plot approval process: genetically engineered plants with plant genes are being field tested while genetically engineered plants with bacterial or fungal genes and genetically engineered bacteria of are still tied up in litigation (Crawford 1986a).

Considering the application of ecological models to the problem, it becomes apparent that the available ecological models are believed to apply universally, but they fit microorganisms much better than macroorganisms. If we look at the models that ecologists have developed to explain the interactions between hares and lynx, or different species of warblers, the assumptions of these models are really better suited to explain the dynamics of much simpler classes of organism than they are for the more complex multi-cellular organisms to which they are usually applied (Andrews and Harris 1986). Thus models for systems of simple organisms, such as viruses and bacteria (or a viral or bacterial pathogen and its host), are more precise than models for larger organisms. Here the short life expectancy of the virus or bacteria and the relative absence of intricacies such as age structure or complex social behavioral fits the conditions of simple differential or difference equation models. Similarly the large amounts of data that are available for things like measles epidemics, allow such models to be more finely tuned than is possible for complex predator prey systems. Both the ease with which variables can be estimated and the correlation between the model's predictions and the observed empirical data are better for micro-organisms than macro-organisms.

The rich background of data and theory suggests that biotechnology can gain much, rather quickly, from interactions between population biologists and molecular biologists. The following sections consider the ecological consequences of the deliberate release of potential competitors and pathogens, the two most important ecological interactions to affect deliberately released organisms, drawing attention to differences in the ecology of macro- and microorganisms and pointing to questions which remain for understanding the environmental impacts of applied biotechnology. After that, cascade or indirect effects are discussed. These are non-obvious consequences of the introduction of organisms into the complex web of interactions that make up natural communities, and a matter of serious concern to ecologists. The final section attempts to draw some more general conclusions from these deliberations.

Macro-organisms: Competition

The results of the release of many macro- and micro-organisms will depend crucially upon competitive interactions. For example, INA- Pseudomonas syringae is intended to be released in such large numbers that it outcompetes (displaces) wild type INA+ bacteria on plants and retards ice formation (Lindow 1985.) Agricultural releases of cultivated plants will not be intended as competitors (except in the market place). However, whether those plants or their traits become established in native ecosystems will depend upon their competitive interactions with native species, although other interactions (see Pathogens below) will be involved as well.

For our purposes here, a broad definition of competition seems appropriate: we define competition as occurring if the organisms limit each other's abundance in the environment without consuming each other. The degree of inhibition may be unequal and many narrower definitions are included within this usage (e.g., Krebs 1985, Ricklefs 1986).

The importance of competition to the success of introduced organisms as reported in the literature provides one type of information on the possible

competitive impact of introduced genetically introduced organisms. However there is a paucity of data. For example, Simberloff (1981) summarized a series of papers on the fate of introduced organisms, and concluded that in only 3of more than 854 introductions was competition primarily responsible for local extinction of a native organism. In an additional 51 cases (6% of the total) he reported that competition between introduced and native species was thought to have occurred, with most introductions listing no effect whatever on native species. However, as many authors have pointed out (Moyle in press, Herbold & Moyle in press, Pimm & Levin this volume, Sharples this volume) most of the papers from which the data were drawn were not written with the impact of the introduction in mind, and so "no mention of effect" is difficult to interpret. We agree with Pimm and Levin (this volume) that there must have been some effect in most cases. However, where impacts are cited, they more frequently focus on habitat destruction and predation than competition, leading to a poorly documented view that competitive problems are not the most likely or most serious of results of introduced species. As far as the data available to us at this time goes, competition is not usually cited as the cause of problems resulting from the introduction of species.

The problem for deliberate release lies in the rare case in which some undesirable response does occur. Clearly competition with aliens CAN result in extinction of native taxa. The three situations to which Simberloff refers are the extinction of native fish by blue and blackchin tilapia (Tilapia aurea and T. melanotheron) in Florida (Courtenay et al. 1974) and the replacement of the black rat (Rattus rattus) by the brown rat R. norvegicus) in Britain (Lever 1979). In addition, competitive interactions between invading and resident ant species have frequently caused extinction of the local ant species (Wilson 1971). Among plants, competition-based local extinction appears relatively common: under the introduced grazing regime of 18th century California, Mediterranean annual grasses (e.g. Avena spp.) outcompeted and replaced native perennial bunch-grasses (e.g. Stipa spp.) (Wagner 1978). A number of unproven cases also exist where a pattern consistent with competitive displacement ("competitive exclusion") is apparent. For example, the spread of the South American lizard Anolis sagrei in southern Florida appears to be at the expense of the similar sized resident A. carolinense (L. Vitt, pers. comm.)

Although extinction is the most dramatic endpoint, interactions which damage ecosystems without driving species to extinction are likely to be unacceptable in many circumstances. Presumably the domination of ecosystems in the southeastern U.S. by kudzu-vine (Pueraria lobata), or Australia by the prickly pear (Opuntia stricta) were such cases. Since virtually all terrestrial plants have very similar requirements (Tilman 1982), the successful invasion of these alien plants can be viewed as successful competition against established native species.

Generally, intact ecosystems resist invasion (Krebs 1985, Chesson 1986, Hubbell and Foster 1986, Ricklefs 1986), but nonetheless, some species have successfully entered healthy native ecosystems, for example Kentucky bluegrass in the tallgrass prairie (Weaver and Fitzpatrick 1934). Apparently most ecosystems contain enough slack that species invade by using under-utilized resources, probably shifting relative abundances, but generally not causing extinctions or major displacements (Smith and Kohler 1977, Simberloff 1981). Something must change (see Cascade section and Pimm and Levin this volume) but often it is not enough to have a detectable impact. The smaller the ecosystem,

the less play there seems to be (for example on islands as opposed to the mainland) probably because the natural fluctuations that occur in population numbers are more likely to reach zero in small groups than in larger ones. (See also Sharples, Pimm and Levin, this volume).

Ecological studies of competitive interactions indicate that these interactions resolve themselves very quickly, so that in general, we see the outcome but rarely the dynamics of the interaction (McNaughton and Wolf 1979, Krebs 1985, Ricklefs 1986).

Theories of competition do not suggest any differences in the impact of competition on macro- and micro-organisms.

Competition for Mutualists

A second form of interaction that has potential to create problems is competition for mutualists. Mutualisms are interactions in which both species benefit: for example, pollination, in which the plant has its gametes transported to another plant and the transporting animal receives food. Mutualisms such as nitrogen fixation and pollination are extremely important to ecosystem function (Boucher et al. 1982).

Many important mutualisms are complex interactions in which several essentially independent species can play each role. Generally the system will include some cheaters, who receive the benefit without returning a benefit, such as nectar thieves in pollination systems. Theory of mutualism (Boucher et al. 1982, Soberon Mainero and Martinez del Rio 1985) concludes that there is no way in which the "cheaters" can be eliminated, but that they are a tolerable cost to a functioning mutualism. However, too many cheaters will destroy a mutualism: either the partners will cease to interact because there was insufficient mutualistic exchange or because one or both of the partners goes extinct for lack of the service the mutualism provided. Such a situation would be hard to detect except during the brief period of mutualistic failure.

The introduction of an alien into mutualistic systems can be serious for the mutualist as well as the outcompeted species. Out-competing one partner does not guarantee that the new partner is a good one. For example, the honey bee, Apis melifera, has replaced many small bees in North America, at least locally. There is little documentation of the pre-Apis system, but presumably some plants have been favored and some reduced by the substitution of pollinators. Apis, for example, often robs small flowers of their nectar without contacting the pollen. Thus it is an effective competitor for nectar but may be a poor pollinator (Faegri and van der Pijl 1979, Inouye 1983).

In South Africa, major rearrangements of species are in progress because one mutualist is being outcompeted, with devastating effects not only on its numbers, but on the numbers of other mutualistic partner. The aggressive, introduced, Argentine ant Iridomyrmex humilis is causing massive reproductive failure of plants in the Proteaceae because the Argentine ant is competitively replacing the native ants these plants relied on to distribute their seeds. The Argentine ant has outcompeted those species for food and nest sites, but it has little interest in seeds, and so does not gather, move or bury seeds of some 170 species of Proteaceae. These seeds are consequently eaten in large numbers by vertebrate herbivores or destroyed by ground fires. In either case

reproductive failure caused the composition of the plant community to change significantly (Bond and Slingsby 1984).

Competitive interactions which cause the displacement of a mutualist probably usually simply replace one mutualist with another, as has been the case across most of the range of the honey bee: it provides adequate pollination for most native American flowers. However, the interaction need not be properly maintained even though an ecologically equivalent organism is introduced, as the above examples indicate. Thus, introductions of species that interact as mutualists, such as rhizobia or plants requiring pollination, should be tested carefully to be sure they are indeed ecologically equivalent to existing partners and so will both receive adequate services and provide adequate services in any mutualisms that are part of their basic biology.

Intraspecific Competition

It is a well established principle in ecology that intraspecific competition is more severe than inter-specific competition (Wilson 1971, Ricklefs 1983, Krebs 1985). Members of the same species have greater potential to reduce each others' numbers by usurping resources than do members of different species. This is because members of a single species generally use the same resources to the same degree in the same ways, while members of different species tend to have at least some differences in resource use and need. From the standpoint of interaction of genetically engineered species in the natural environment, this means there is greater potential for the introduced form, if it has a competitive advantage, to drive the preexisting closely related form to extinction. Inter-specific competitive interactions more often result in the better competitor driving the poorer one out of all habitats that the better competitor can physically tolerate, but there are usually sites where the better competitor cannot go and there the poorer competitor will persist. For variants within the same species, there may be no place the poorer competitor can live where the better cannot. This will again lead to extinction. For example, in England, where introduced mammals had native counterparts, such as squirrels, there was ecological replacement of the local animal, e.g., the red squirrel, by the introduced gray squirrel (Lever, 1979). In contrast, there do not seem to have been resident mammals that were affected by the introduction of the coypu (Myocastor coypus, a large South American aquatic rodent) or the polecat Mustela putorius furo. The pattern of replacement also occurred for rats, the brown rat, Rattus norvegicus replacing the black rat R. rattus (Lever 1979). Thus, although little impact is seen for animals without close relatives, replacement occurred when very similar species are introduced.

Within a species, competition is intense (Ricklefs 1983, Krebs 1985). The dark "melanistic" moths of Biston betularia replaced the previous normal, gray form across the midlands of England within 80 years in response to industrial pollution of the habitat and changed the color of moth resting sites. This is of course usually viewed as an example of adaptive changes in populations. However, seen from another angle, it demonstrates that, when given the appropriate selective advantage, one genetic form can effectively replace a pre-existing one. Granted alleles were simply substituted in an evolving population, but different individuals possessed those alleles, and the "normal, native" genotype that previously represented the species at that site became rare to nonexistent.

Thus, the minor changes in existing organisms that are the preferences of biotechnological release are the ones with the greatest likelihood for extremely rapid and potentially complete replacement of existing forms. Where there is genetic exchange between the groups, the loss of the initial alleles can occur without substitution at other loci, and need not require complete loss of the alleles (genes) to replace the character (depending on the dominance relationships between the alleles). However, for micro-organisms, or any species that are carefully engineered not to exchange genes with their local relatives, the replacement must be complete, with the whole suite of genes in each type proliferating or vanishing depending on the net selective difference between the two.

Consequences of Competition

Most historically reported examples of competition have not resulted in extinction, and most introductions have had no visible effect on the natural communities. The cases in which a competitor eliminated unrelated species are generally ones in which the introduced species was a generalist in some major characteristic, particularly food habits. For example, weedy ants tend to have competitive interactions ending in (local) extinction, even where they are of a very different size. Similarly, fish interactions which lead to extinction (Courtenay et al. 1974) are usually cases where species with broad general feeding habits eliminate others. Similarly, competition between plant species is widespread and important because most plants share broad water and nutrient requirements, allowing a generalists competitor like brome (Bromus spp.) or bamboo to usurp space previously used by a diversity of other species. One recommendation to reduce these risks is to introduce specialists.

However, the prospect for competition leading to extinction are much greater for closely than distantly related species, and replacement rather than single gene substitution must occur if the two competitors cannot cross. This means that slightly modified organisms should receive especial scrutiny.

Specific recommendations for avoiding undesirable results of competition from an introduction are to determine the presence of competitors and compare the introduced species in an enclosed experimental trial. If there is coexistence in this experiment, there will almost certainly be coexistence in the vastly more complex natural system. If there is competitive exclusion of the native endemic species then further studies need to be done to determine whether the diversity and spatial heterogeneity of the natural systems will allow coexistence. There is a vast literature in ecology and evolutionary biology on the dynamics of competitive interactions and the conditions for coexistence of potential competitors (Tilman 1982, Krebs 1985, Chesson 1986). These could be worked into a series of protocols if the case warranted it.

The literature referred to above chiefly concerns macro-organisms, although some major papers (Hutchinson 1961, Tilman 1982) have considered competition in micro-organisms and the principles are universal. One operational difference is that the less known about the species and ecosystem, the harder it is to delineate competitors. Thus, at our present state of knowledge, we may find it easier to design field trials that are appropriate for an macro-organism introduction than for that of a micro-organism. The principles are believed to be the same however.

Pathogens and Biological Control Agents

Although the release of any form of pathogenic organism as a biological control agent is likely to be a major contentious issue in the use of biotechnology, it is also an area where a solid mathematical framework exists which can be used to examine the properties of a pathogen before it is released. It is therefore an area where interactions between population biologists and molecular biologists should be encouraged at all levels of development. Two types of benefits are likely to arise from these interactions.

1) The efficiency of the proposed biological control agent could be improved considerably by defining the ecological conditions which determine its transmission and growth rate.

2) Potential side effects could be determined in the field trials designed as a series of controlled experiments which 'dissect' the system and suggest improvements in the design of the pathogen, before it is used on a wider commercial scale.

Before examining these two points in more detail let us briefly contemplate some simple models for pathogens in host populations and consider the relevance of these models to the release of artificially produced pathogens. The models are discussed in more detail in Anderson & May (1981) and in Anderson (1982). Figure 1 illustrates a flow chart for a simple pathogen which when released into a population causes increases in the mortality rate of infected hosts. The 'flow' of uninfected and infected individuals through the population is given in the simple two equation model

$$dX/dt = a(X+Y) - bX - \beta XY \qquad (1)$$

$$dY/dt = \beta XY - (\alpha + b)Y \qquad (2).$$

Here a is the birth rate of new hosts, b is the death rate of uninfected hosts, β is the transmission rate of the control agent (pathogen) and α is the increase in mortality resulting from the introduction of this pathogen. If the total number of hosts, H, is given by $H = X + Y$, then we may define the prevalence of infection, y, as the proportion of hosts infected, Y/N. We can thus obtain a third equation for the change of host population density with time

$$dH/dt = rH - \alpha Y \qquad (3).$$

Here we have defined the intrinsic growth rate of the hosts, r, as $r = a-b$. Equations 1-3 can be rearranged to give an expression for the basic reproductive rate of the pathogen, R

$$R = \beta H/(\alpha + b) \qquad (4).$$

Thus R will be the expected number of secondary infections a single infection is capable of producing, and is thus directly analogous to the term, R0, used by ecologists to describe the 'expected number of offspring' produced by free-living animals and plants. This concept of basic reproductive rate is central to any understanding of the circumstances under which pathogens persist within

host populations. The qualitative nature of any host-pathogen relationship depends only on the magnitude of R.

If $R > 1$, the infection persists within the host population, and the prevalence approaches a steady value of

$$y = 1 - 1/R \qquad (5).$$

Conversely, if $R < 1$, the disease cannot persist within the host population. The condition $R > 1$ for maintenance of the infection suggests that the host population must exceed a threshold density if the pathogen is to be able to establish (Kermack & McKendrick 1927, Anderson & May 1979, May & Anderson 1979). For the system of equations given above, the threshold density is defined as

$$HT = (\alpha + b)/\beta \qquad (6).$$

When host density falls below this level the pathogen prevalence will start to decrease and will eventually go extinct. Figure 2 illustrates an example of a threshold density for pathogen establishment in a simple laboratory system studied by Anderson (1957).

Several biological conclusions are illustrated in the relationship shown in equation (6). First, pathogens with low transmission rates can only persist within high density host populations, while pathogens with high transmission efficiency can persist in lower density host populations. However, pathogens with low transmission efficiency can persist in low density host populations if the hosts are relatively long-lived (that is if natural and pathogen-induced mortality rates are low). Finally highly pathogenic control agents (high) will only persist in relatively high-density host populations.

Specifically, if the introduced pathogen is to control the host or pest population, then its pathogenicity has to exceed the growth rate of the host population, formally

$$\alpha > r \qquad (7).$$

Under these conditions it regulates the host population at a stable equilibrium level, H*. This disease controlled equilibrium population size is

$$H^* = \frac{\alpha\,(\alpha + b)}{\beta\,(\alpha - r)} \qquad (8).$$

or equivalently

$$H^* = \frac{\alpha\, HT}{(\alpha - r)} \qquad (9).$$

Notice that the controlled population, H* must exceed HT, the threshold number of hosts, (although if $\alpha \gg r$, then $H^* \simeq HT$). The equilibrium number of hosts infected at equilibrium, y*, is given by

$$y^* = r / \alpha \quad (10).$$

Notice that the prevalence of infection varies inversely with the pathogenicity of the control agent. The relation between the pathogenicity of the host and the equilibrium host population density and prevalence are illustrated in Figure 3. Note that maximum levels of reduction in host density are produced by pathogens of intermediate pathogenicity.

If α is less r, then the pathogenicity of the parasite is insufficient to regulate the density of the host population. In this case, the host population tends to grow exponentially at a rate $\sqrt{}$, which is less than r:

$$\sqrt{} = r - \alpha \quad (11).$$

This exponential growth of the host population is essentially a feature of the absence of any form of density-dependent regulation, other than the pathogen, in the model. In reality, other factors, such as resource limitation will limit population growth. Such effects can readily be incorporated into the framework (Anderson & May 1981).

We can consider two further qualities of a potential pathogen within the above framework. Initially let us consider a potential control agent which also reduces the fecundity of infected hosts. This effect may be incorporated by taking the birth rate of infected hosts to be a(1-f). Here $f(1 \geq f \geq 0)$ measures the severity of the parasite's effect on host reproduction: f=1 corresponds to elimination of all reproduction by infected hosts; f=0 is the opposite extreme where there is no effect. Equations 1 - 3 may thus be re-written:

$$dX/dt = a(X+Y) - bX - \beta XY - faY \quad (12)$$

$$dY/dt = \beta XY - (\alpha + b)Y \quad (13)$$

$$dH/dt = rH - (\alpha + fa)Y \quad (14).$$

In this model the basic reproductive rate of the pathogen, R, and the threshold density for establishment, HT, remain unaltered from the values given in equations 4 & 6 respectively. If the introduced pathogen is also able to reduce the fecundity of infected hosts to a level a(1-f), then the control is established provided

$$\alpha > [a(1-f) - b] \quad (15).$$

Thus less pathogenic strains of the control agent are required to regulate the host.

Figure 4 illustrates the effects of pathogen induced reductions in host fecundity on H* and y*. In general, both H* and y* are lower than when pathogens have no effect on host reproduction (f=0). Pathogens which reduce host fecundity are thus likely to be better control agents than parasites that simply reduce host survival.

A second important effect occurs when the pathogen may be transmitted vertically to the offspring of the hosts either before or just after they are born. This is a common feature of many viral pathogens of insects. To incorporate vertical transmission into the basic model (eqns 1-3), we assume that a fraction q ($1 \geq q \geq 0$) of the offspring of infected hosts pass directly into the infected class. The resulting model may be written

$$dX/dt = a(X+Y) - bX - \beta XY - aqY \qquad (16)$$

$$dY/dt = \beta XY - (\alpha + b - aq)Y \qquad (17)$$

$$dH/dt = rH - \alpha Y \qquad (18).$$

The basic biological considerations of the parasite's reproductive rate, R, and the host threshold density, HT, are now modified by the ability of a single infected host to produce secondary cases by vertical as well as by horizontal transmission. The threshold density for pathogen establishment is now

$$HT = (\alpha + b - aq)/\beta \qquad (19).$$

Thus the presence of vertical transmission tends to lower HT, making it easier for the pathogen to establish and persist at low host population densities. Similar effects occur if we consider the equilibrium host population density, H*, after introduction of the pathogen. Again the pathogen can only regulate the host when $\alpha > r$, as in the two previous models. providing that this inequality is met then

$$H^* = \frac{\alpha(\alpha + b - aq)}{\beta(\alpha - r)} \qquad (20).$$

Notice that it is possible for the pathogen to become established at arbitrarily low levels, when $aq > (\alpha + b)$, however when this occurs it is unable to regulate the host population as $r > \alpha$. It is thus plain that vertical transmission is a "key consideration" when considering the release or design of a potential pathogen. This insight also emphasizes the utility of the modelling aproach adopted here.

"Designer" Pathogens and Biological control

The design of efficient biological control agents is thus an area in which the most immediate advances can be made by cooperation between population and molecular biologists. As an example consider the design of a pathogen to control a pest species. Although many workers might initially consider the optimal biological control agent to be a highly pathogenic micro-organism that operates much as a pesticide does, by instantly killing the target pest organism. However the models outlined above indicate that considerable increases in efficiency accrue if control agents of intermediate pathogenicity are utilized (Anderson 1979,1982; Anderson & May 1981). The argument can be inverted to suggest that the release of apparently innocuous pathogens can lead to large decreases in the population density of organisms infected. This counter- intuitive result is highly pertinent to any discussion of the release of modified pathogens. Although this effect will be beneficial when only the

'target' organisms are effected, it may well be disastrous if other 'non-target' organisms become infected.

Two further considerations should be briefly mentioned in this section on biotechnology and the use of 'designer' pathogens as biological control agents. Essentially, two types of pathogen are likely to be used: the simplest will be highly pathogenic and applied in much the same way as pesticides are applied; the second class will consist of less pathogenic organisms which may be applied once and then left to establish and regulate the hosts at some economically acceptable control density. In both cases it is essential that the pathogen be highly specific to the organism it is targeted against. The ability to affect more than one species may create all manner of complications which we will discuss further below.

In the case where the pathogen is applied much in the manner of a "pesticide", we can use the mathematical framework outlined above to determine the rate at which the pathogen has to be applied in order to eradicate the target organism. Anderson (1982) describes the mathematics of this in lucid detail. The most important result of his analysis is that there exists a critical application rate above which eradication is possible and below which control is relatively ineffective (Fig 5). Estimating the level of this critical density is something that may require quite detailed field trials; but it can certainly be done. Again, interactions between population biologists and biotechnologists can only be beneficial at this stage of the development of a 'designer' pathogen.

In the case where a pathogen is released and allowed to establish in a host population, an important consideration we have not yet addressed is the life expectancy of the transmission stages of the control pathogen. If the pathogen has sufficiently long lived transmission stages then the pathogen can persist in the environment during the occasional periods when the host population drops below the threshold density for establishment. This is likely to be important in a host that lives in a highly seasonal environment. However, if the pathogen has very long lived free-living stages and is fairly highly pathogenic it is likely to cause long term non-seasonal oscillations in the abundance of the pest species (Anderson 1982; Anderson & May 1981). These are likely to be disastrous in situations where sustained economic yields are required. Such effects will also be important when the pathogen affects other non-target organisms.

In short, the population dynamic interactions between pathogens and their hosts may well give rise to complex patterns of dynamic behavior. Interactions between biotechnologists and population biologists in the design of field trials and the tuning of "life history characteristics" for "designer" pathogens can only lead to large increases in the efficiency and safety with which these organisms are developed and tested. Interactions should therefore be encouraged at all levels of funding and administration.

It similarly seems likely that the variation seen in the different genetic strains of many pathogens, may become a feature of some strains of released pathogen. It thus seems sensible to suggest that some form of legislation be developed which might limit the reproductive rate of released potential pathogens. This might serve to partly reduce the rate at which new mutants appear. As this would also reduce Ro, some balance would have to be struck

between the efficacy of the pathogen as a biological control agent and our ability to control the control agent if it got out of hand.

Cascade Effects

One of the more serious problems in environmental impact of deliberately released organisms is the possibility of "cascade effects." These are changes in one species which destabilize other species, leading to complex changes in species far removed from the original disturbance. The cascade effects that the release of genetically engineered organisms might initiate are the least predictable aspects of release. Although ecologists have spent a lot of time examining the structure of ecological communities, it is still rarely possible to predict the ramifications of introducing or removing a seemingly innocuous species from a community. This is mainly because the interactions which link the species within the community operate in a highly non-linear fashion and species interact indirectly with species on different levels in the food web. Thus small changes in the numbers of one species can often cause quite large changes in the density of apparently unconnected species.

Details of how ecologists attempt to model the complex dynamics of food webs are given in Pimm (1982). Although no two food webs are identical, they do possess some commonalities. However, each has its own idiosyncracies and to extrapolate from one to another would be as naive as to assume that the genome of a parasitic nematode, such as the human hookworm, Ascaris lumbricoides, was essentially the same as that of the well studied free-living nematode Caenorhabditis elegans. Large parts of the two will be similar, but some will be very different. Determining the full complexities of any food web is in every way as challenging a task as unravelling the complete genetic code for any specific organism. Here we briefly consider a couple of examples which illustrate how the presence, or absence, of a particular species may cause perturbations which affect species in other parts of the food web. To continue the theme of the earlier parts of this paper, we will first consider examples of introductions of macro-organisms and then consider examples that deal with micro-organisms.

Competitor (or Macro-organism) Introductions and Cascade Effects

One example is given in Figure 7. This is a standard chart of a terrestrial food web, taken from an introductory ecology text book (Ricklefs 1976). Suppose for the sake of argument we wish to control a problem weed by releasing large numbers of a grasshopper that eats only one plant. Specialist grasshoppers exist but do not generally have weed species as their food plants, so a grasshopper might be engineered eats mush thistle instead of Louisiana sagebrush. If we increase the number of grasshoppers, we will (a) decrease the amount of total plant material as there will be increased herbivory, (b) raise the number of grasshopper sparrows, spiders and robber-flies because of the increased amount of food available, and (c) probably increase the number of hawks and other insect-eating birds as they respond to increases in their prey animals. Reduction of the thistles may well lead to an increase in the nontarget plants, due to a reduction in competition for resources. This may in turn lead to increases in the numbers of other herbivores, few of which specialize on thistle, so they benefit from increases in their food plants. However, species for whom thistles are an important part of their diet should decrease as would those species who suffer increased

losses due to grasshopper predators switching to alternate food sources. Essentially then the results of a comparatively straightforward introduction may be quite complex.

A well-documented example of the cascade effect occurred in the Chagres River and Lake Gatun, Panama (Zaret and Paine 1973). Here, the introduction of Cichla ocellaris, the "peacock bass", a medium sized predatory fish, caused massive changes in the structure of the community (Fig 8). This predator fed on the younger stages of planktivorous fish, reducing the density of three species and eliminating seven others. However, there were further-reaching effects; with the reduction of the planktivorous fish, zooplankton densities changed greatly, and forms that were previously severely limited by fish increased in numbers. Similarly, insects, and in particular mosquitos, appeared to increase, this seems to be primarily due to a reduction in predation on their larvae. Furthermore, the vertebrate predators, such as tarpon (coming in from the oceans), terns, kingfishers and herons, which fished heavily in these waters before the spread of Cichla, became much less common and appeared to forage elsewhere in search of food. Were Cichla to be found in all the local waterways, the total populations of the birds would probably be seriously reduced, through death or emigration. Thus, the whole aquatic ecosystem and some of the terrestrial ecosystem has been affected by the introduction of this single species.

A second tale comes from the World Health Organization (Levin and Kimball 1984). In the process of controlling insect pests, DDT was freely applied in Borneo. Houseflies were killed. However, gekkos which fed on the flies, including dead ones, were affected by DDT and died. Dying gekkos were more easily caught by house cats, which received greater concentrations of DDT, sickened and died. The death of the cats resulted in an outbreak of rats which spread bubonic plague and destroyed food stores. While this story is directly caused by passing a toxin along the food chain, which should not happen in biotechnology, it could have occurred by drastic reductions of fly numbers causing drastic reductions of gekkos, which in turn caused drastic reduction of cat numbers and consequent increases in a second prey item of cats.

Pathogen (Micro-organism) Introduction and Cascade Effects

The herds of wildebeest (Connochaetes taurinus), zebra (Equus burchelli) and buffalo (Syncerus caffer) that annually migrate across the Serengeti plains of Tanzania form part of a complex multi-species predator-prey system (Sinclair & Norton Griffiths 1979; Talbot & Talbot 1963). Although ecologists have concentrated on studying the social organization of the different species which interact as predators and prey in this system, one of the most important factors determining the numbers of different species has been the viral disease rinderpest. This viral pathogen was first introduced to sub-Saharan Africa in 1889, when it was brought into Somaliland by cattle imported to feed the Italian army. The infection spread rapidly among the indigenous ruminants; south of the Zambezi the disease is estimated to have attained mortality rates of 90%, killing over five million buffalo, antelopes and other ruminants. Although subsequent epidemics caused lower rates of mortality, the pathogen was still a severe cause of mortality in ungulates until the introduction of a vaccine for domestic ungulates.

The decrease in rinderpest incidence in domestic ungulates led to declines in the rinderpest incidence in wild ungulates and their numbers increased to a higher level (Fig 8). Although this increase in prey density was followed by an increase in the numbers of some species of predators, in particular lions (Panthera leo) and hyaenas (Crocuta crocuta), the numbers of hunting dogs (Lycaon pictus) declined (Fig 8). While several explanations have been proposed to explain this apparent paradox, Hanby & Bygott (1979) suggest that increased competition with hyaenas may be responsible for the decline in hunting dog numbers, other evidence suggests that a reduction in exposure to rinderpest has lead to increased mortality of hunting dog pups from distemper (Schaller 1972, Dobson & Hudson 1986). Canine distemper is closely related to rinderpest and the two viruses may illustrate cross- immunity to each other. Whichever of these two mechanisms is operating, and they are not mutually exclusive, they illustrate the complex ways in which the constituent members of 'many-species' systems interact with each other to produce changes in population density which are not only counter-intuitive, but often completely unpredictable.

A mathematical framework has been developed by Holt & Pickering (1986) which examines the consequences of a pathogen asymmetrically affecting one of a pair of competing species. These models suggest that the ability of a parasite to reverse the competitive asymmetries between species may also cause large changes in the density of a species not affected by a pathogen. These considerations will again be important when pathogens are released under the naive assumption that they will only change the density of the species they directly infect.

Not all cascade effects are unpredictable: a good understanding of the functional connections within the community, such as diet breadth of herbivores and the degree to which predators control numbers of herbivores in a system will indicate the direction of some changes. In Fig 7, the meadowlarks and praying mantises which eat grasshoppers are shown as not eating other insects (unlikely!) so other species should not suffer increased predation if meadowlark and mantis numbers increase. However, an understanding of the impact of annual variation on food web connections would be required in many cases where meteorological conditions create differences between years. In the simplified example of Fig 7, it would make a big difference to know that in dry years voles use the musk thistle as a source of water, while in wet years they utilize other food sources. Their switch from competing with grasshoppers for thistle plants to benefitting from the removal of a plant that competes with their food plants depends entirely on the available water. Although these more complex interactions are known in a few well studied ecological communities, they have not been studied in any but a handful such systems. Obviously caution must be extended if modified organisms are going to be used as control agents in complex ecosystems. However, a lot can be learned about the structure of a community if a few well designed experiments are undertaken and again interactions between ecologists and biotechnologists can only be of mutual benefit in this area.

Cascade effects argue strongly first for the use of field tests that are as realistic and as complete as possible. They also argue for greater caution in the consideration of introductions that are aimed at intact ecosystems, when compared to those for agricultural or veterinary situations. In both the latter cases, the "ecosystem" may be sufficiently well controlled that "mopping up"

operations are tenable if something goes seriously wrong. Neither comparable levels of knowledge nor control exist for natural ecosystems (forests, rivers, the ocean) and we certainly have limited ability to call back organisms released into these ecosystems.

Conclusions

In this paper we have discussed the various ways introduced organisms may interact with other novel organisms in the environment. We have chiefly concentrated on the effects of introduced species as competitors and pathogens. We see enormous potential for the use of "artificially manufactured" organisms in the areas of pest control and increased food production. However, we strongly feel that major advances can be made in the guidelines for the release of organisms and their effective use, if more interactions are initiated between biotechnologists and population biologists.

The major biological differences we see between macro-organisms (loosely animals and plants) and microorganisms (loosely fungi, bacteria, and viruses) occur as a consequence of the different sizes of these organisms. Microorganisms are by definition much smaller. Size will affect the impact of the introduction, in the sense that larger introduced organisms move more biomass, cycle more nutrients, and are seen by more people than smaller introduced organisms. Whereas, there are a million Pseudomonas syringae on a single pea leaf, a million wild sunflowers would require areas of the order of a county or more. Although the introduction of small organisms will be less noted than even a few big organisms in the wrong place, they are potentially more of a problem to control as their rapid rates of reproduction may allow them to proliferate and spread through the environment more rapidly.

This brings us to one of the most serious aspects of introducing organisms. Micro-organisms are likely to mutate and speciate much more rapidly than macro-organisms. All the environmental impact research is rapidly wasted if the introduced taxa evolve characteristics they lacked when they were introduced. The potential for evolution has three aspects: the genetic system, the number of organisms and the selection pressure applied.

The genetic system considerations include especially whether individuals can cross, exchanging genes, and whether genes are exchanged via plasmids and viruses within or between species. They also consider the breeding system, since species that require two individuals of opposite sexes for reproduction do not as readily colonize distant areas as species in which a single individual can establish a breeding population. The dangers of evolutionary lability will be seriously considered in any deliberate release. Microorganisms appear to recombine less as a general rule, but to exchange genes with more distantly related organisms than is the case in macro-organisms. However, those generalizations are best applied to the well-studied species of micro- and macro-organisms: among wild and little known species, there are diverse breeding systems in both groups. Plants and some vertebrates are known to exchange genes with members of other genera, while bacteria in the field may do little recombining of any sort. Thus, we feel that the key to genetic lability is not so much differences between macro and microorganisms but specific to particular organisms.

Neither group is ever perfectly adapted to its environment, given real space and finite populations so any organism is likely to evolve if presented with an environmental opportunity (Colwell 1984).

As mentioned above, we do feel that the differences in the numbers of macro- and microorganisms utilized in any release, make the chance of an evolutionary change much more likely in the microorganisms. If you have an event that happens once in 107 and there are 108 bacteria released, it has a good chance of occurring, but is unlikely among the 105 corn plants. Mutations, particular recombinations, etc. are more probable in larger numbers of organisms, other things being equal, and, almost always, one is dealing with many more microorganisms.

Finally, selection pressure is a key to evolutionary change. If a particular trait increases survival or reproduction, it can be expected to increase in frequency in the population. We do not see any basic differences between how selection would act on released micro or macro-organisms. Some kinds of release are more favorable for selection on the released organisms than others. These differences stem mainly from the various uses of engineered organisms, not from any inherent differences in the two types of organisms. Our only caveat, is that again, sheer numbers can come into play. Severe selection that reduces 99% of a species will leave behind 10 survivors of a population of 1000, and 10 thousand survivors of a population of a million. The survivors of such selection are likely to include at least some better adapted individuals: the population of 10 thousand is more likely to be a viable one than the group of 10. Thus, selective change and adaptive response are better in larger populations under selection, other things being equal. Again, then, microorganism populations will be more likely to evolve in response to environmental selection pressures than are smaller macro-organism populations.

We feel that there are interactions in which concern about the impact of introduced organisms on the environment is merited. These concerns relate directly to differences between micro and macro-organisms in the way these are expressed. The differences are not so much those of basic biology, because both groups include very diverse types of organisms with very different potentials for invasion and establishment, but rather in the number of organisms of each type that are found in an area. The vastly greater numbers of microorganisms make them more capable of evolutionary change. On the other hand, the vastly smaller size of microorganisms means that in the great majority of cases, they may slip quietly into the ecosystem without doing any harm at all, the smaller microorganisms will be unremarked and totally ignored by everyone except specialists looking for them.

In conclusion, we suggest that problems related to the size of organisms are crucial to the design of rules and regulations for their release. Far more is known about macro-organisms than is known about microorganisms, so that less effort will be required to give a sensible evaluation of the risks associated with the release of a "new" macro-organism. However, unless active cooperation is encouraged, both financially and "politically", between microbiologists and population ecologists for additional research on microorganisms in natural ecosystems, this area of biotechnology will remain dominated by unsubstantiated speculation. Meeting this challenge is perhaps the most important next step if

the vast potential of biotechnology is to be brought to bear in reducing the world's problems of disease, pests and hunger.

Literature Cited

1. Alexander, M. and Andow, D.A., "Fate and Movement of Microorganisms in the Environment Part 1. Survival and Growth of Bacteria, Part 2. Dispersal of Microorganisms with Emphasis on Bacteria," Environ. Management. 10:463-494, 1986.
2. Anderson, E.S., The Relations of Bacteriophages to Bacterial Ecology Microbial Ecology. Seventh Symposium of the Society of General Microbiology, (London: Cambridge University Press, 1957).
3. Anderson, R.M., "Parasite Pathogenicity and the Depression of Host Population Equilibria," Nature 279:150-152, 1979.
4. Anderson, R.M., Theoretical Basis for the Use of Pathogens as Biological Control Agents of Pest Species, Parasitology 84:3-33, 1982.
5. Anderson, R.M. & May, R.M., "Population Biology of Infectious Diseases. Part I," Nature 280:361-367, 1979.
6. Anderson, R.M. & May, R.M., "The Population Dynamics of Microparasites and their Invertebrate Hosts," Philosophical Transactions of the Royal Society of London 291:451-524, 1981.
7. Anderson, R.M. & May, R.M., "Vaccination Against Rubella and Measles: Quantitative Investigations of Different Policies,"Journal of Hygiene 90:259-325, 1982.
8. Andrews, J.H. & Harris, R.F. "r- and K-selection and Microbial Ecology," Advances in Microbial Ecology 9:99-147, 1986.
9. Baldwin, R.E., Hawaii's Poisonous Plants (Hilo, HI: The Pteroglyph Press, 1979).
10. Bond, W. and Slingsby, P., "Collapse of an Ant-plant Mutualism: the Argentine Ant (Iridomyrmex humilis) and Myrmecochorous Proteaceae," Ecology 65:1031-1037, 1984.
11. Boucher, D.S., James, S. and Keeler, K.H., "The Ecology of Mutualism," Ann. Rev. Ecol. Syst. 13:315-347, 1982.
12. Cairns, J. Jr. and Pratt, J.R., "Ecological Consequence Assessment: Effects of Bioengineered Organisms," Water Resources Bull. 22:171-182, 1986.
13. Chesson, P.L. "Environmental Variation and the Coexistence of Species," Community Ecology, J. Diamond & T.J. Case (eds.) (New York: Harper & Row, 1986).
14. Colwell, R.K., "Biological Response to Perturbation: Genome to Evolution," Engineered Organisms in the Environment: Scientific Issues, H.O. Halvorson, D. Pramer & M. Rogel (eds.) (Washington DC: American Society for Microbiology, 1984).
15. Corbet, G.B., "The Distribution of Mammals in Historic Times," The Changing Flora and Fauna of Britain, Syst. Association Special Vol #6, D.L. Hawksworth (ed.) (New York: Academic Press, 1974).
16. Courtenay, W.R. Jr. and Robins, C.R., "Exotic Aquatic Organisms in Florida with Emphasis on Fishes: a Review and Recommendations," Trans. Amer. Fish Soc. 102:1-12, 1973.
17. Courtenay, W.R. Jr., Sahlman, H.F., Miley, W.W. II and Herrera, D.J. "Exotic Fishes in Fresh and Brakish Water of Florida, "Biol. Conserv. 6:291-302, 1974.
18. Crawford, M., "Regulatory Tangle Snarles Agricultural Research in the Biotechnology Arena," Science 234:275-277, 1986a.

19. Crawford, M., "Researcher Reprimanded for Pseudorabies Test," Science 234:667-668,1986b.
20. DeWet, J.M., Harlan, J. and J.R., "Weeds and Domesticates: Evolution in the Manmade World," Econ. Bot. 29:99-107, 1975.
21. Dietz, K., "The Incidence of Infectious Diseases under the Influence of Seasonal Fluctuations," Lecture Notes in Biomathematics 11:1-15, 1976.
22. Dobson, A.P. & Hudson, P.J., "Parasites, Disease and the Structure of Ecological Communities," Trends in Ecology & Evolution 1:11-14, 1986.
23. Elton, C.S., The Ecology of Invasion by Animals and Plants (New York: John Wiley and Sons, 1958).
24. Faegri, K. and van der Pijl, L., The Principles of Pollination Ecology, Third Edition (Oxford, England: Pergamon Press, 1979).
25. Grime, J.P., Plant Strategies and Vegetation Processes (New York: John Wiley and Sons, 1979).
26. Hanby, J.P. & Bygott, J.D., "Population Changes in Lion and Other Predators," Serengeti. Dynamics of an Ecosystem, A.R.E. Sinclair & M. Norton-Griffiths (eds.) (Chicago: University of Chicago Press, 1979).
27. Harris, P.D. Peschken and Milroy, J., "The Status of Biological Control of the Weed Hypericum perforatum in British Columbia," Can. Entomol. 101:1-15, 1969.
28. Hawksworth, D.L. (ed.), The Changing Flora and Fauna of Britain, Syst. Assn. Special Vol #6 (New York: Academic Press, 1974).
29. Holt, R.D. & Pickering, J., "Infectious Disease and Species Coexistence: a Model of Lotka-Volterra Form," American Naturalist 126:196-211,1985.
30. Hubbell, S.P. & Foster, R.B., "Biology, Chance, and History and the Structure of Tropical Rain Forest Tree Communities," Community Ecology, J. Diamond & T.J. Case (eds.) (New York: Harper & Row, 1986).
31. Huffaker, C.B. and Kennett, C.E., "A Ten-year Study of Vegetational Change Associated with Biological Control of Klamath Weed," J. Range Manag. 12:69--82, 1959.
32. Hutchinson, G.E., "The Paradox of the Plankton," Amer. Nat. 95:137-145, 1961.
33. Inouye, D.W., "The Ecology of Nectar Robbing," The Biology of Nectaries, B. Bentley and T. Elias (eds.) (New York: Columbia University Press, 1983).
34. Kermack, W.O. and McKendrick, A.G., "A Contribution to the Mathematical Theory of Epidemics," Proceedings of the Royal Society A. 115:13-23, 1927.
35. Krebs, C.J., Ecology. The Experimental Study of Distribution and Abundance, Third Edition (New York: Harper and Row, 1985).
36. Lever, C., The Naturalized Animals of the British Isles (Granada, St. Albans: Herts, 1979).
37. Levin, S.A. and Kimball K.D. (eds.), "New Perspectives in Ecotoxicology," Env. Manag. 8:375-442, 1984.
38. Lewis, W.H. and Elvin-Lewis, M.P.F., Medical Botany. Plants Affecting Man's Health (New York: John Wiley and Sons, 1977).
39. Lindow, S.E., "Ecology of Psudomonas syringae Relevant to the Use of Ice-deletion Mutants Constructed in vitro for Plant Frost Control," Engineered Organisms in the Environment: Scientific Issues, H.O. Halvorsen, D. Pramer & M. Rogul (eds.) (Washington, DC: American Society for Microbiology, 1985).
40. MacArthur, R.H., and Wilson, E.O., The Theory of Island Biogeography (Princeton, NJ: Princeton University Press, 1967).
41. May, R.M. & Anderson, R.M., "Population Biology of Infectious Diseases: Part II," Nature 280:455-461, 1979.
42. McNaughton, S.J. and Wolf, L.L., General Ecology Second Edition (New York: Holt, Rinehart and Winston, 1979).

43 Oka, H.I. and Morishima, H., "Ecological Genetics and the Evolution of Weeds, Biology and Ecology of Weeds, W. Holtzner and N. Numata (eds.) (the Hague: Dr. W. Junk, Publishers, 1982).
44. Pimm, S.L., Food Webs (London: Chapman & Hall, 1982).
45. Regal, P.J. "Models of Genetically-engineered Organisms and their Ecological Impact," Ecology of Biological Invasions in North America and Hawaii, H.A. Mooney (ed.) (New York: Springer-Verlag, 1986).
46. Ricklefs, R.E. The Economy of Nature. A Text Book in Basic Ecology (Portland, OR: Chiron Press, 1976).
47. Ricklefs, R.E., Ecology, Third Edition (New York: Chiron Press, 1983).
48. Schaller, G.B., The Serengeti Lion. A Study of Predator-Prey Relations (Chicago: University of Chicago Press, 1972).
49. Sharples, F.E., "Spread of Organisms with Novel Genotypes: Thoughts from an Ecological Perspective," Recomb. DNA Tech. Bull. 6:43-56, 1982.
50. Simberloff, D.S., "Community Effects of Introduced Species," Biotic Crises in Ecological and Evolutionary Time, M.H. Nitecki (ed.) (New York: Academic Press, 1981).
51. Soberon Mainero, J., and Martinez del Rio, C., "Cheating and Taking Advantage in Mutualistic Associations. The Biology of Mutualism," Evolution and Ecology, D.H. Boucher (ed.) (London: Croom Helm Ltd., 1986).
52. Smith, H.M. and Kohler, A.J., "A Survey of Herpetological Introductions in the United States and Canada," Trans. Kansas Acad. Sci. 80:1-24, 1977).
53. Stern, A.M., "Potential Impacts of Environmental Releases of Biotechnology Products: Assessment Regulation and Research Needs. Applications," Environmental Management 10:445-453, 1986.
54. Teich, A.H., Levin, M.A. and Pace, J.H., Biotechnology and the Environment. Risk & Regulation (Washington, DC: Amer. Assoc. Adv. Science, 1985).
55. Tilman, D., Resource Competition and Community Structure (Princeton, NJ: Princeton University Press, 1982).
56. Vidaver, A.K., "Plant-associated Agricultural Applications of Genetically Engineered Microorganisms: Projections and Constraints," Recomb. DNA Tech. Bull. 8:97-102, 1985.
57. Wagner, R.H., Environment and Man Third Edition (New York: W.W. Norton and Co., 1978).
58. Weaver, J.E. and Fitzpatrick, T.J., "The Prairie," Ecol. Monogr. 4:109-295, 1934.
59. Wilson, E.O., The Insect Societies (Cambridge, MA: Belknap Press, 1971).
60. Wilson, E.O., Sociobiology (Cambridge, MA: Belknap Press, 1975). 61. Zaret, T.M. and Paine, R.T., "Species Introduction in a Tropical Lake," Science 182:449-455, 1973.

FIGURE LEGENDS

Figure 1. Flow chart of a simple model for a host population into which an pathogen is introduced. Two classes of hosts are distinguished, uninfected but susceptible, Xt, and infected, Yt. The various birth and death rates are as described in the main text.

Figure 2. A growing population of Salmonella typhi (Vi-type A) infected with the phage virus Vi-phage A (data from Anderson 1957; redrawn from Anderson & May 1981). Notice that the phage density does not 'take off' until the bacterial host population exceeds some threshold value.

Figure 3. These two graphs illustrate the impact of the rate of pathogen-induced mortality, , on the equilibrium number of hosts, H* (graph A), and the equilibrium prevalence, y* (graph b). In the shaded regions the pathogen is unable to regulate population growth rate since < r. (after Anderson & May 1981).

Figure 4. These graphs are similar to Figure 3 except that the pathogen was assumed to have no effect on host survival but to simply suppress host survival. The rate of infected host reproduction is denoted by a(1-f), where 'a' is the birth rate of uninfected hosts and f records the severity of the pathogen's impact on fecundity. The impact of the value of 'f' on host density, H* (graph A) and the prevalence of infection, y* (graph B) are illustrated. In the shaded region the pathogen is unable to regulate host population growth since the rate of infected host reproduction, a(1-f), exceeds the rate of mortality b. The critical value, f', at which regulation is possible is given by f' = (1 - b/a).

Figure 5. The impact of the rate of introduction of 'pesticide' like pathogen on the equilibrium density of hosts, H* (graph A) and the equilibrium prevalence of disease, y* (graph B). Note that H* and y* only begin to show a significant decline as A approaches the critical value Ac (after Anderson 1982).

Figure 6. This figure illustrates the relation between the proportion of a population host that have to be immunized (at or near birth) to eradicate an infection, and the basic reproductive rate R of the infection. Alternatively p may be expressed as a function of A; this situation is shown for L=70 and L=35 years. The infection is eradicated for values of p in the shaded area and it persists otherwise (after Anderson & May, 1983)

Figure 7. Some of the feeding relationships in a simple food web (after Ricklefs 1976).

Figure 8. Numbers of Wildebeest (dots and error bars) and Hunting dogs (open triangles) in the Serengeti. Also illustrated are the percentage of Wildebeest and Buffalo infected with antibodies to rinderpest (circles and diamonds). The left scale gives the prevalence of infection. The right scale shows the density of wildebeest and wild dogs (after Dobson & Hudson 1986).

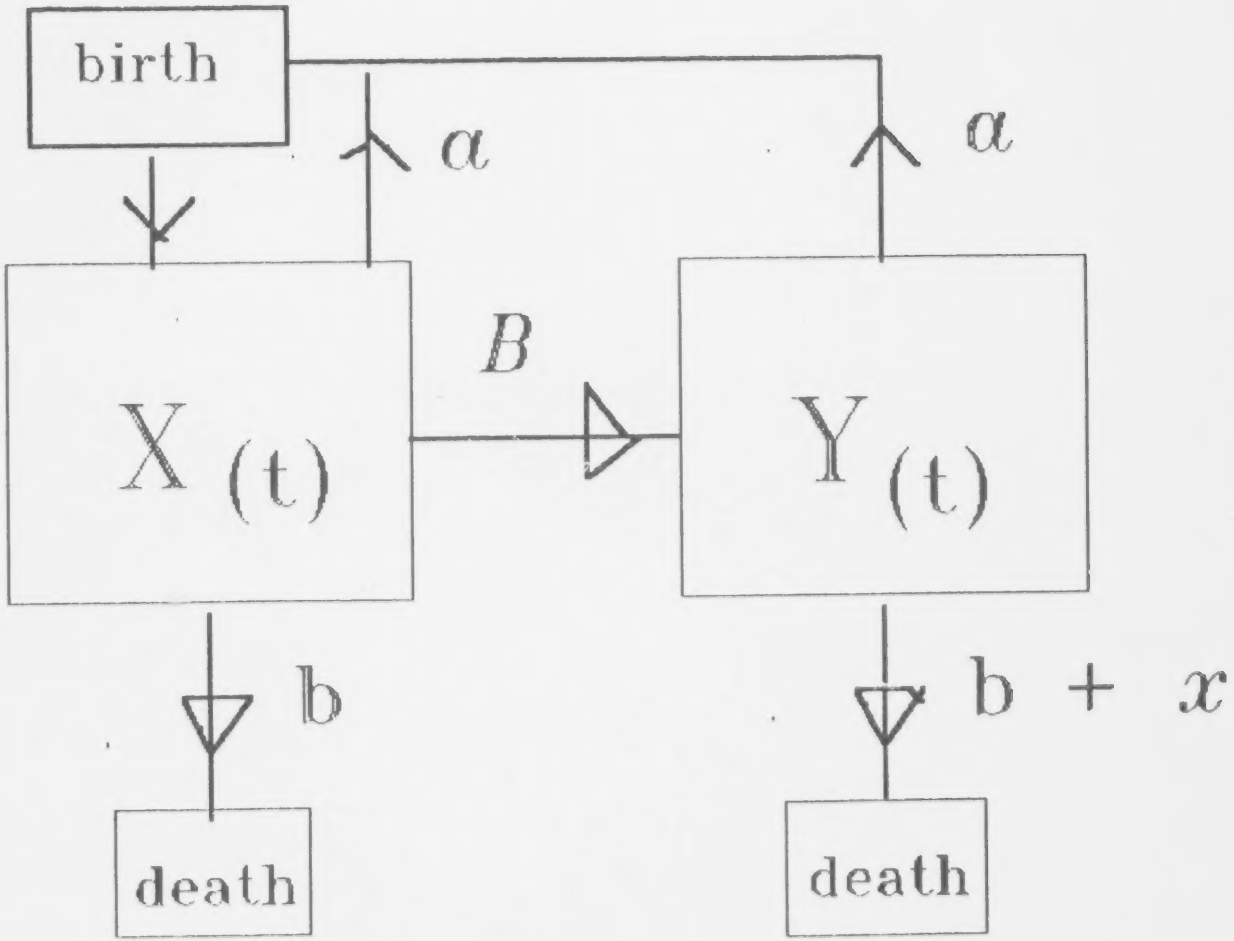


Figure 1. Simple host – pathogen model.

Figure 2.

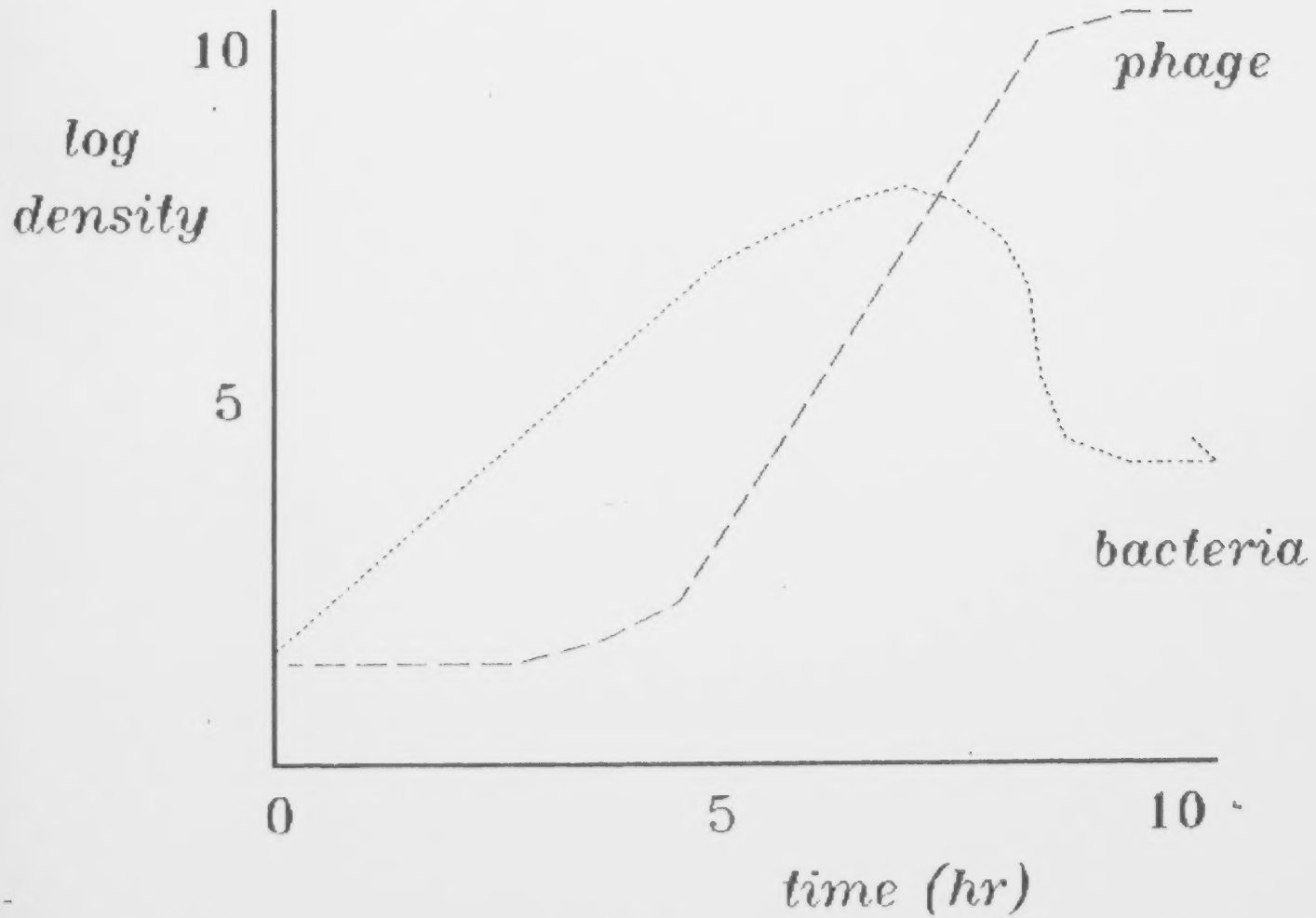
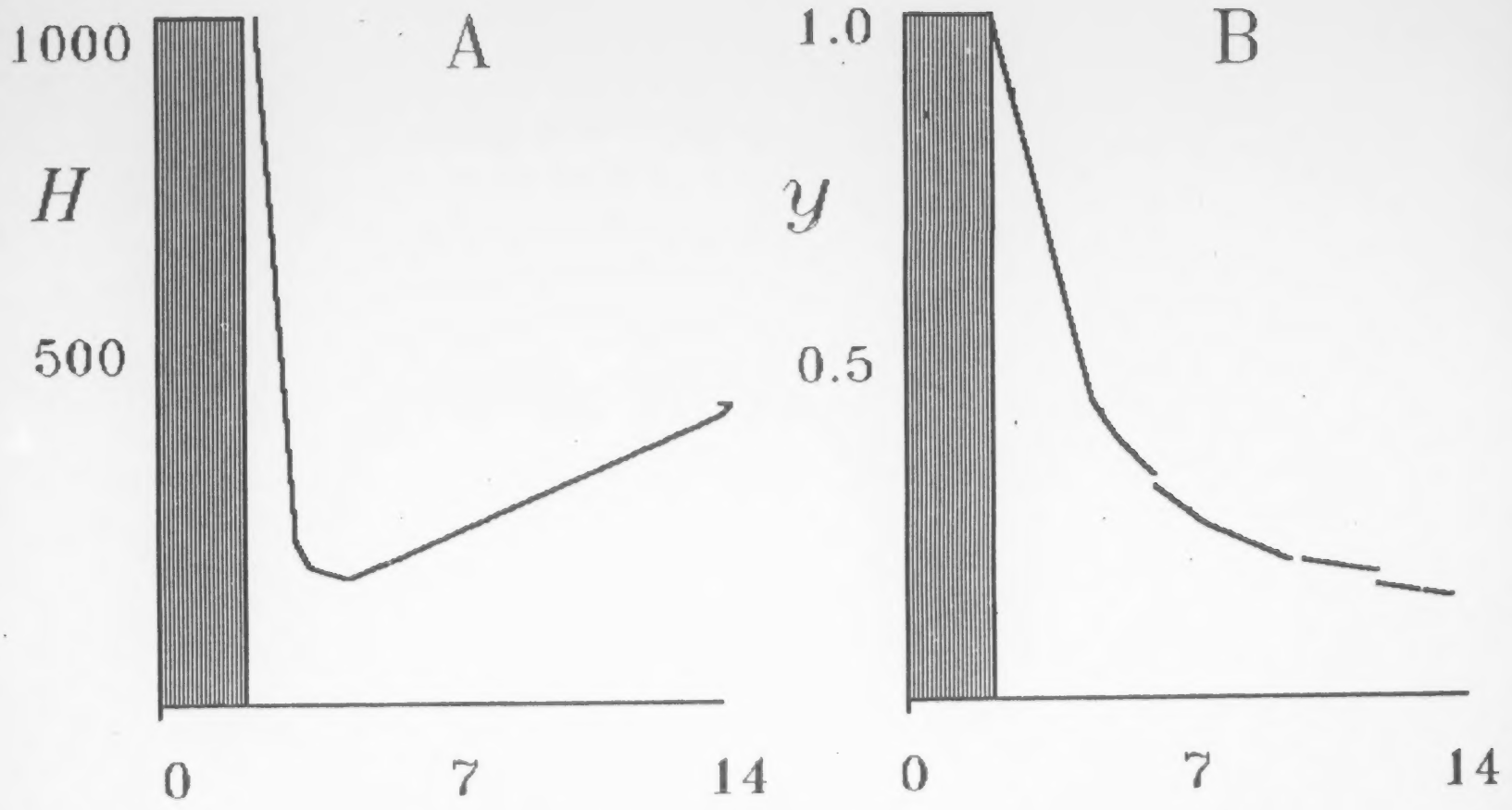


Figure 3.



Rate of pathogen induced host mortality, a

Figure 4

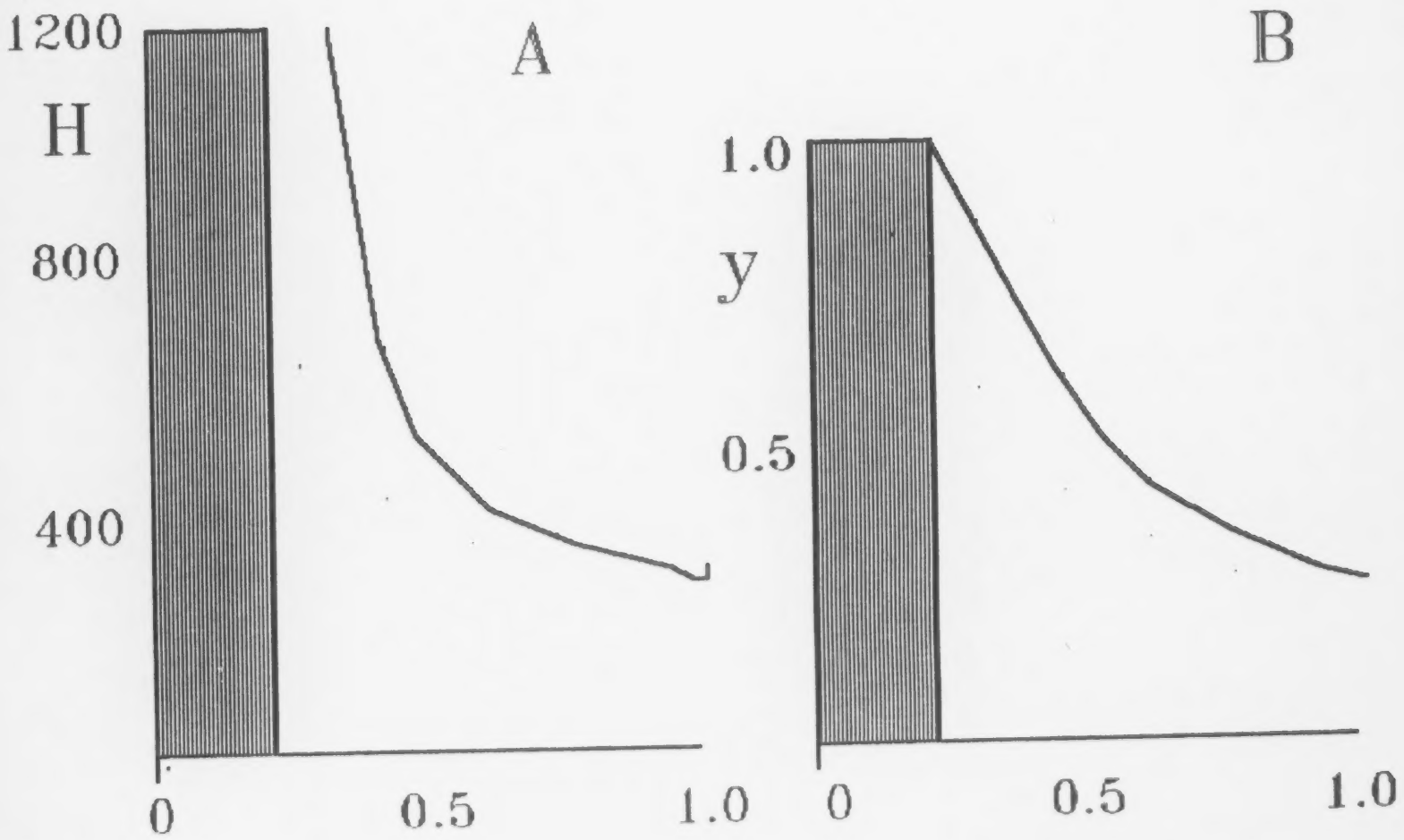

1200
H
800
400
A
0
0.5
1.0
B
1.0
y
0.5
0
0.5
1.0
Reduction in fecundity of infected hosts, f.

Fig 5

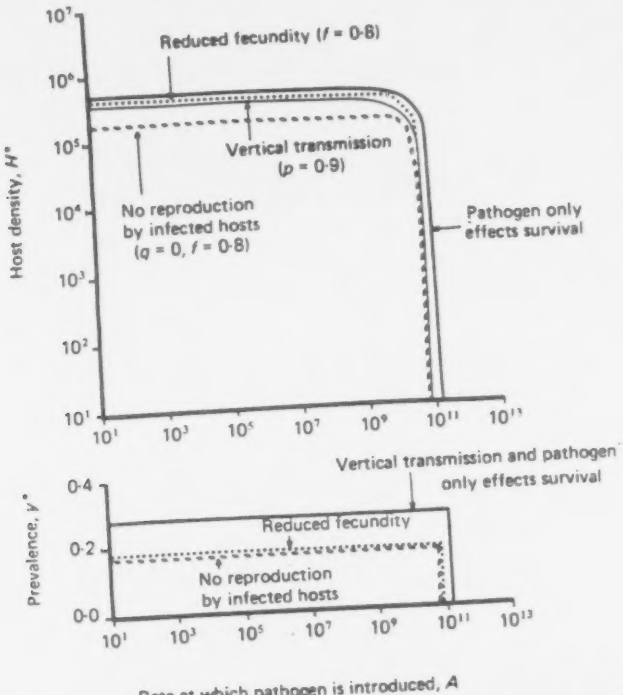

Reduced fecundity (f = 0·8)
Vertical transmission (p = 0·9)
No reproduction by infected hosts (q = 0, f = 0·8)
Pathogen only effects survival
Host density, H*
Vertical transmission and pathogen only effects survival
Reduced fecundity
No reproduction by infected hosts
Prevalence, y*
Rate at which pathogen is introduced, A

Figure 5.

Fig. 13. The impact of the rate, A, at which the pathogen is introduced on the equilibrium density of hosts, H^* (graph A) and the equilibrium prevalence of disease, y^* (graph B). In graph A, the thick solid line denotes the case represented by Fig. 9 where the pathogen is horizontally transmitted and solely effects host survival; the dotted line is the case represented in Fig. 11 where the pathogen is horizontally transmitted and reduces host fecundity and survival ($f = 0{\cdot}8$); the thin solid line is the case represented in Fig. 10, where the pathogen only influences host survival but is vertically and horizontally transmitted ($q = 0{\cdot}9$). The dashed line denotes a new case where the pathogen is transmitted horizontally, has no influence on host survival ($\alpha = 0$) and entirely eliminates infected host reproduction ($f = 1$). Note that in all cases H^* only begins to show a significant decline (over the situation where $A = 0$) as A approaches the critical value A_c. In graph B the dotted and dashed lines are as defined above but the solid line incorporates the thick and thin solid lines of graph A. The prevalence remains constant over all values of A until the host is exterminated. (Parameter values as for Fig. 9 except $\alpha = 9{\cdot}0$/year and $r = 2{\cdot}5$/year.)

with a period of roughly 6–8 years (the precise period depending on the specific pathogenicity of the virus) and exhibits a series of important features, some of which are often observed in natural systems. First, note that in the absence of the disease, the host population would grow exponentially. Second, the peak in disease prevalence within the pest population occurs shortly after the peak in host abundance. Thirdly, the pathogen regularly reduces the host population below the critical density, H_T, necessary for disease persistence. During such periods the pathogen persists as a free-living infective stage in the forest environment, although absent from the host population. It is a mistake to conclude that the

reproductive rate R', is $R' = R(1-p)$ with R as defined in Equation (1.7) (Dietz, 1974; Smith, 1970). The condition $R' < 1$ then gives Equation (1.14). The relation between p and R is illustrated in Fig. 1.6. If R is large, the proportion that must be immunized approaches unity. If other things are equal (such as the efficiency of a vaccine, or the ease of its application within the population) diseases with high R values will be much more difficult to control than those with low values. The effective equivalence between the basic reproductive rate R and the average age at infection, A, defined in Equation (1.12), is further illustrated in Fig. 1.6 where Equation (1.12) is used to re-scale the x-axis to give a relation between p and A for a specified value of L. In other words diseases which have a low average age at infection will be more difficult to control than those with a high average age at infection.

Immunization programmes clearly act to reduce the value of R and hence theory predicts they will tend to increase, both the inter-epidemic period, T, and the average age at infection, A (see Equations (1.12) and (1.13)).

For example, in England and Wales, as a consequence of widescale immunization, the average inter-epidemic period for measles has increased

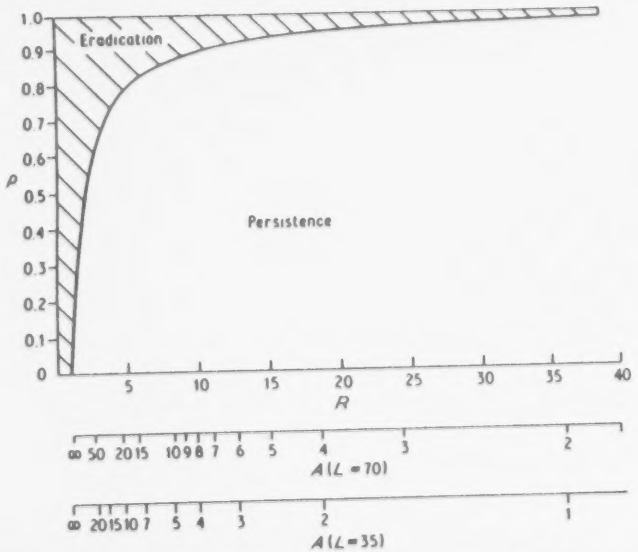


Figure 1.6 This figure illustrates the relation, Equation (1.14), between the proportion, p, of a community that must be immunized (at or near birth) to eradicate an infection, and the basic reproductive rate R. Alternatively p may be expressed as a function of A (using Equation (1.12)); this relationship is shown for $L = 70$ and $L = 35$ years. The infection is eradicated for values of p in the shaded area and it persists otherwise (*Anderson* and May, 1982a).

from 2.2 to 2.6 years, and for whooping cough it has increased fron years (Anderson and May, 1982a). The frequency distributions of inte periods for both diseases, before and after the introduction of imm are displayed in Fig. 1.7. The epidemiology of rubella in the United good example of the impact of immunization on the average ag individuals acquire infection. As documented in Table 1.6, the intro

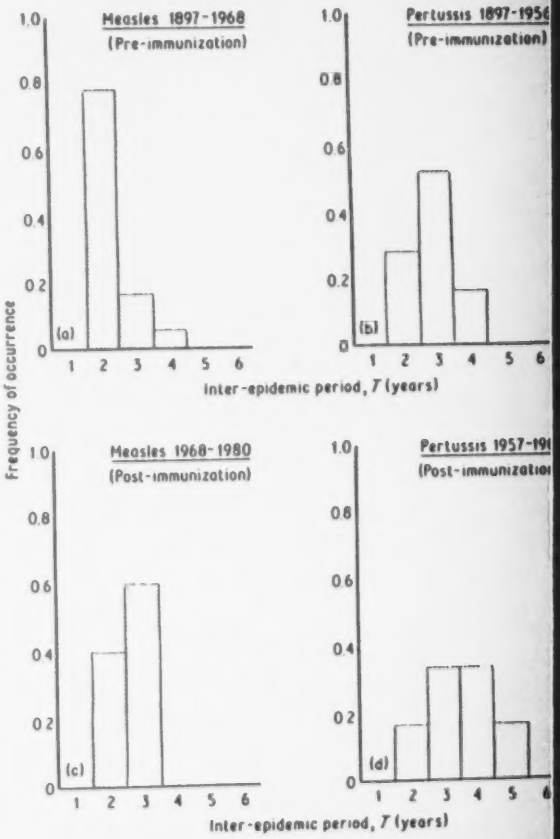


Figure 1.7 This figure shows the frequency distributions of the i period, T, for measles and whooping cough in England and Wales. (a) Me the introduction of immunization; 1897–1968. (b) Pertussis prior to the in immunization; 1897–1956. (c) Measles after the introduction of i 1968–1980. (d) Pertussis after the advent of immunization, 1957–19 periods for graphs (a), (b), (c) and (d) are 2.2, 2.8, 2.6 and 3.5 years resp from the Registrar General's statistical review of England and Wales.

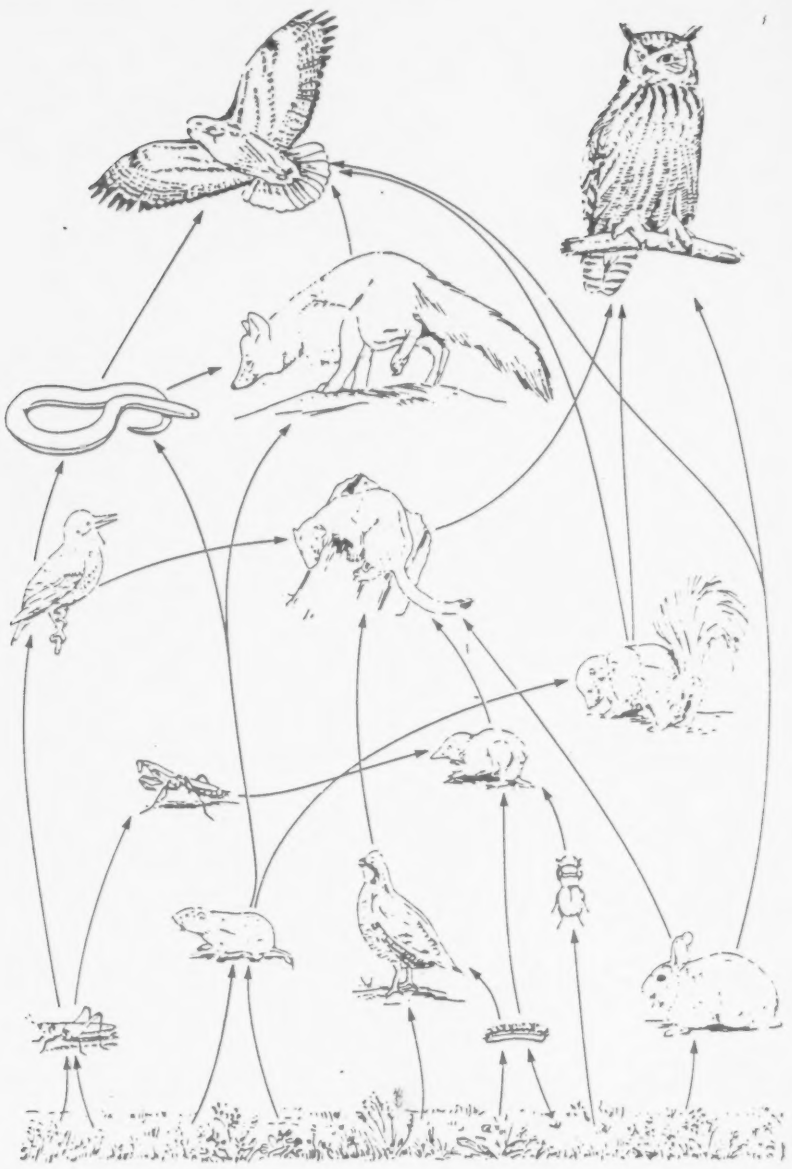


FIGURE 8-2 Some of the feeding relationships in a simple food web.

Figure 7

Rickleft 1976

collections comparing the Trinidad
n, where *Cichla* has not yet invaded,
where the fish community repre-
ts the pre-*Cichla* conditions, with
rro Colorado Island, where the *Cich-*
population has recently peaked (Ta-
2). In the Trinidad Arm region, the
e we chose was relatively shallow
5 meters), with dense vegetation,
ainly *Hydrilla* sp., lining the shore.
t this site, we made a large semicircle
(30 m by 5 m) with a 1.27-cm mesh
nylon net. The net was attached to the
shore, with the ends 10 m apart, to
form the letter "D." The second site,
along the shores of Barro Colorado Is-
land, was a comparable cove, depth ≤ 5
m, with the same species of dense vege-
tation lining the banks and choking
much of the waters. A 30 m by 5 m
nylon net closed off the mouth of the
cove, leaving an area approximately 30
m by 25 m. The results are presented
in Table 2. The Barro Colorado Island
and shoreline several times that of the
Trinidad Arm site. A census of the
fishes at each site had been taken pre-
viously by direct observation.

The Trinidad Arm community is composed of 14 fish species, 11 of which, in terms of their percentage of the biomass, contribute significantly. These are the genera *Melaniris*, *Astyanax*, *Compsura*, *Pseudocheirodon*, *Roeboides*, *Aequidens*, *Cichlasoma*, *Neetroplus*, *Gobiomorus*, *Gambusia*, and *Poecilia*. In contrast, the Barro Colorado Island site shows seven fish species present, but dominated heavily, in terms of percentage of biomass, by *Cichla* and *Cichlasoma*. This comparison indicates (excluding *Hoplias*) that, of the 11 previously important species, *Cichla* has completely eliminated seven and has reduced three others. One, *Cichlasoma*, has apparently increased. [The increase in *Cichlasoma* is probably due to the elimination by *Cichla* of species that formerly fed on the *Cichlasoma* fry, thereby seriously limiting the population. *Eleotris*, although abundant numerically, is a very small species, most of those we found being ≤ 2 cm SL. The role of this fish in the lake is not understood and is omitted from further discussion, although it seems that *Cichla*'s presence is resulting in an increase of this smaller species. The presence of a substantial number of *Roeboides* came as a surprise because this species was never seen during our numerous diving activities. *Roeboides* primarily feeds by ingesting scales of other fishes (*13*) and apparently has survived *Cichla* by remaining in the midst of the dense aquatic foilage where it must await other fishes darting in for cover.] The results from these fish collections basically support the generalizations we made after several years of observing the associations of *Cichla* with the native fish communities.

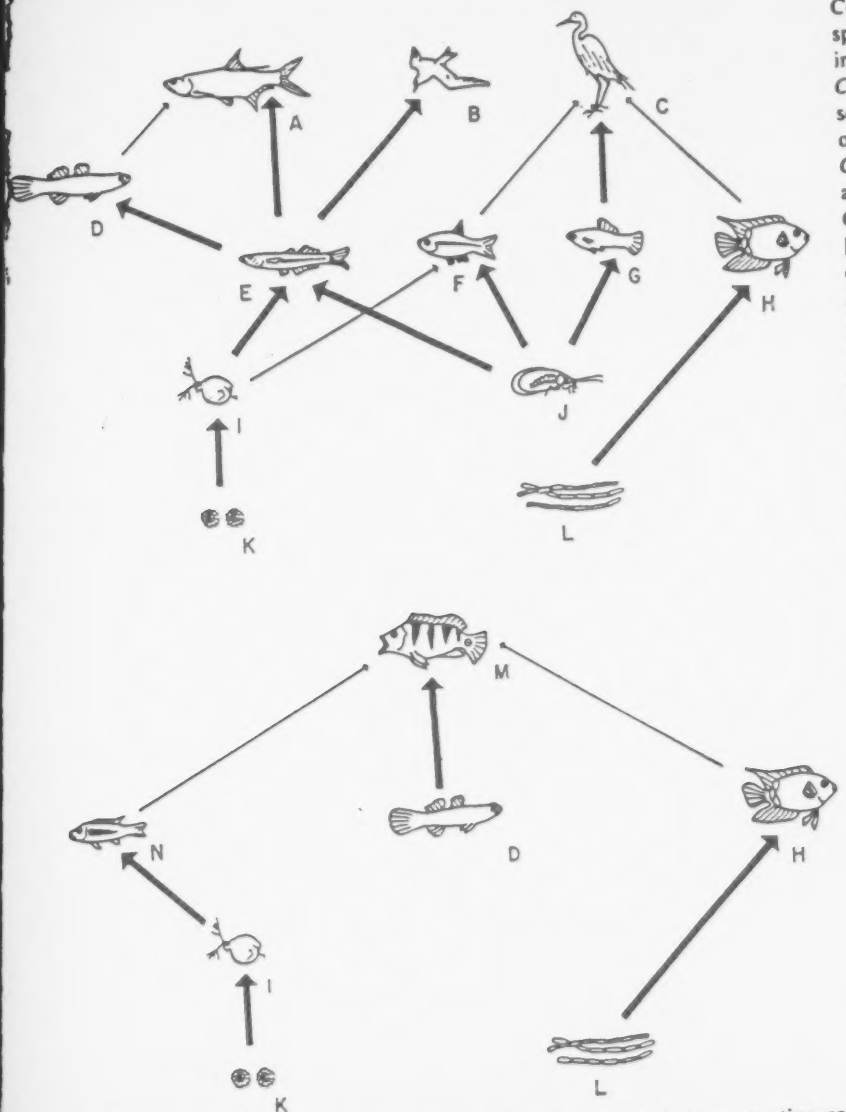


Fig. 7. Generalized food webs of common Gatun Lake populations, contrasting pre-*Cichla* (or present non-*Cichla*) regions (top) with *Cichla* regions (bottom). Thick arrows indicate that food item is of major importance to predator or herbivore, thin arrows indicate minor importance. Key to species: (A) *Tarpon atlanticus*; (B) *Chlidonias niger*; (C) several species of herons and kingfishers; (D) *Gobiomorus dormitor*, (E) *Melaniris chagresi*; (F) characinidae, including four common species: (G) poeciliidae, including two common species—one exclusively herbivorous, *Poecilia mexicana*, and one exclusively insectivorous, *Gambusia nicaraguagensis*; (H) *Cichlasoma maculicauda*; (I) zooplankton; (J) terrestrial insects; (K) nannophytoplankton; (L) filamentous green algae; (M) adult *Cichla ocellaris*; (N) young *Cichla*.

Cichla's Effect on the Food Web

Cichla predation affects most directly the secondary consumers, and these changes are most easily observed. However, these secondary consumers are related to and dependent upon other lake populations, and thus any change in the population of prey fishes must result in second- and third-order effects throughout other trophic levels. Figure 3 presents a generalized view of the trophic structure of Gatun Lake, based

Zaret and Paine 1973

Figure 7.

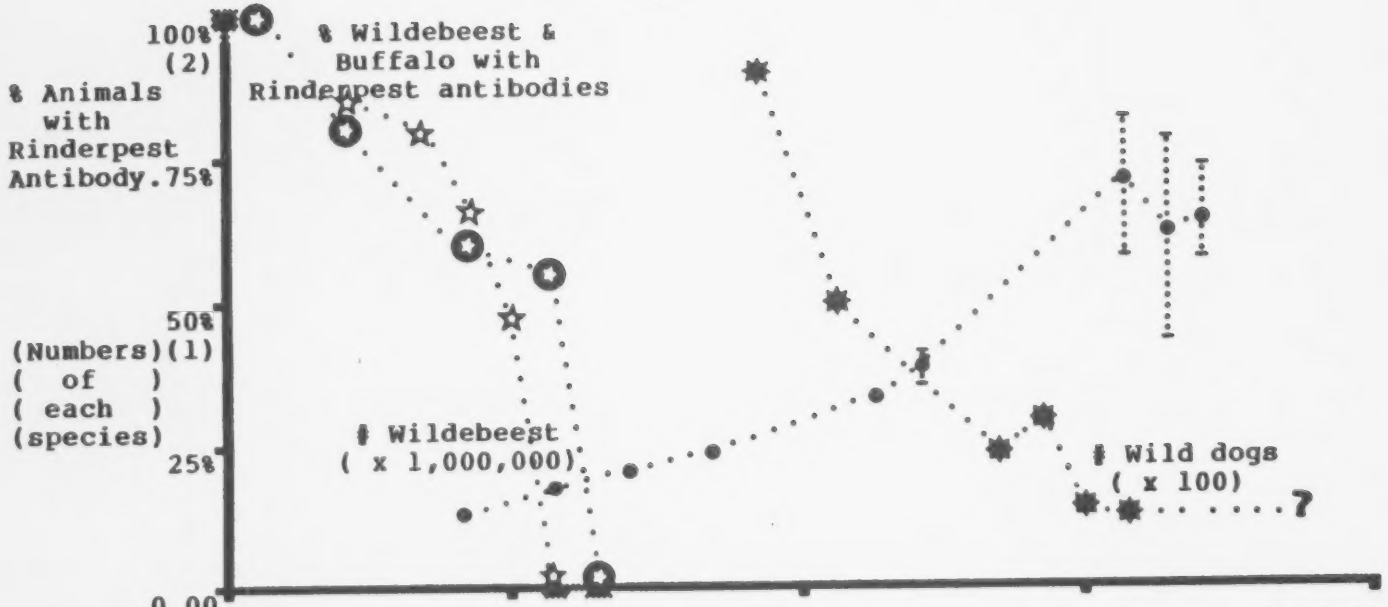

100%
(2)
% Animals with Rinderpest Antibody.
75%
50%
(Numbers) (1)
(of)
(each)
(species)
25%
0.00
% Wildebeest & Buffalo with Rinderpest antibodies
# Wildebeest (x 1,000,000)
# Wild dogs (x 100)
?
1955.00
1962.00
1969.00
1976.00
1983.00
Date

FIGURE 8.

TABLE 1. Environmental Concerns.

1. Released organisms kill or injure people.

2. Released organisms kill or injure animals or plants used by people.

3. Released organism disrupts natural community.

TABLE 2. Characteristics Affecting Potential for Evolution of Released Organisms.

1. Genetic system of released organisms.

Introductions of diverse genes increases the chances of extinction.

a) Can they cross with other organisms?

Are genes transferred via plasmids or viruses?

Do they undergo meiosis?

b) Are two sexes required for breeding or can single individuals start a population?

2. Number of organisms released.

The more released, the greater chance of evolution.

3. Selection pressure on released organisms.

The greater the selection pressure, the more likely that the organisms evolve.

ARE THERE SPECIFIC GENETIC MODIFICATIONS THAT ARE INHERENTLY SO SAFE AS TO NEED NO REVIEW?

Richard E. Lenski
Department of Ecology and Evolutionary Biology
University of California, Irvine

and

Conrad A. Istock
Department of Ecology and Evolutionary Biology
University of Arizona

All concerns about the deliberate release of genetically engineered organisms into the environment must focus on three interrelated questions. (1) What will be the fate of the genetically engineered organisms in nature? (2) What will be the effects of the genetically engineered functions on the environment, including effects within ecological communities which have been established either by nature or by humans? (3) Will the engineered genetic material be transferred to other organisms present in the environment?

An engineered organism can be viewed as being inherently safe if the following can be demonstrated by, or strongly inferred from, experimental tests: (i) the engineered organisms will not survive and reproduce after their release into the environment; (ii) the engineered genetic, physiological, and ecological functions will have no lasting adverse effects on the environment; (iii) and the engineered genetic material will not be transferred to any other organism.

If any one of the statements (i), (ii), or (iii) above is not true there is some likelihood that a given release of genetically engineered organisms may have an undesirable impact on the environment. For example, engineered genetic material which has been transferred in situ to some other organism may have a different ecological fate, or function, than those intended when the engineered organism was first made and released.

In the interest of statutory and regulatory effectiveness, it can be asked whether any categories of genetic modifications can be found for which statements (i), (ii) and (iii) will be invariably true, so that no review prior to deliberate release of the engineered organism into the environment is necessary. It can be similarly asked whether any categories of genetic modifications can be identified for which either (i) or (ii) or (iii) is likely not to be true, so that a detailed review on a case-by-case basis is obviously prudent.

The Coordinated Framework for Regulation of Biotechnology has recently set forth criteria for the level of review that is to be required prior to the deliberate release of an engineered organism into the environment. In this Framework, several categories of genetic modifications are specifically identified as being either high or low risk, and thus deserving of, respectively, either a detailed or an abbreviated review. We will comment on the usefulness and validity of the categories set forth in this Framework, and suggest additional categories that may be of use in revising these criteria.

Crop Plants and Domesticated Animals

Genetically engineered crop plants and domesticated animals appear, in general, to be likely candidates for safe release into the environment. However, there may be some important exceptions. Particular attention in screening for these exceptions should be given to two key factors: whether it has been demonstrated that gene flow to related wild organisms is or is not possible; and whether or not the engineered crop plants or domesticated animals have relatives that are problematic as weed plants or feral animals.

Especially close attention should also be paid to crop plants, or domesticated animals, which have been radically altered in some fundamental metabolic function, say in the case of plants by the incorporation of genes permitting a plant to fix nitrogen or mobilize insoluble nutrients when it does not ordinarily do so. Increased freshwater pollution from augmented nutrient flows could result. With more experience, however, we expect this category of biotechnology to be deemed quite safe.

Pathogens and Pests

Genetically engineered organisms which are made from known pathogens or pests, or use genetic material from such organisms, certainly qualify for detailed review on the basis of their potentially adverse effects on non-target organisms. However, a sharp dichotomy cannot be drawn between "outright" and "opportunistic" pathogens, the latter being suggested for exemption from close scrutiny under the Coordinated Framework.

The distinction between outright and opportunistic pathogens ,or pests, is an artificial dichotomy forced out of a continuum over which pathogenic organisms range from those capable of initiating and pursuing a virulent infection in a healthy host organism to those capable of infecting only host organisms that have been compromised by prior injury, malnourishment, infection, or the like. This continuum is widely recognized throughout discussions of "host specificity" and "degrees of virulence". In fact, it is reasonable to presume that outright pathogens evolve from opportunistic pathogens, which in turn have been derived from non-pathogens. According to Stanier et al (1963, p. 561): "Any organism that gains the ability to invade the host tissues has a tremendous advantage, since invasion brings it into direct contact with the rich supply of nutrients in the host's tissues and frees it from competition with the other members of the normal flora. Many common pathogens may have evolved in this way; the virulent forms of corynebacteria (diphtheria), streptococci (pneumonia), and neisseriae (gonorrhoea and epidemic meningitis), for example, all have their harmless counterparts among the organisms of the normal flora." When humans, another animal or a crop plant, become the specific target host of interest, viruses and fungi also often have their virulent, moderately infective, and non-pathogenic relatives.

The ease with which the virulence characteristics of opportunistic and outright pathogens can be altered by genetic changes is quite striking. Most Escherichia coli are harmless members of the intestinal flora, although they are also frequently opportunistic pathogens, causing a variety of nosocomial (hospital-acquired) infections. Changes in the surface antigens of E. coli,

which may occur by alterations of single genes, can determine whether or not a particular strain is virulent (Williams Smith and Huggins, 1980). Shigella spp., in contrast, are outright pathogens, capable of invasive growth in the enteric environment, thereby causing dysentery. However, detailed population studies have revealed that Shigella spp. "are probably not sufficiently distinct from E. coli to warrant generic or even specific status" (Selander et al 1986: p. 16). Rapid changes in virulence characteristics have also been documented for viral and fungal pathogens. Temperate viruses that infect bacteria may be rendered virulent by changes at only one or a few genetic loci (Levin and Lenski 1985). The myxoma virus was introduced for the biological control of rabbits, which had become pests after their introduction into Australia, but the myxoma virus has undergone a pronounced reduction in virulence since its introduction (May and Anderson 1983). Fungal pathogens of plants often evolve virulence characteristics against new cultivatable varieties within a few years after the new cultivars are widely planted (Van der Plank 1968).

Change in the environment of a pathogen, or its introduction into a new environment, may have dramatic effects on its degree of virulence. Artificial and disturbed environments seem particularly likely to result in an effective increase in the virulence of a pathogen. Legionella pneumophila, the causative agent of the Legionnaire's disease, occurs in natural freshwater habitats, but it is also adaptable to life in cooling towers and other man- made aquatic environments from which it has easier access to human hosts (Selander et al 1985). The preventative use of antibiotics may actually increase the likelihood of serious infections, because it "creates an ecological vacuum in the gastrointestinal tract that opens the way for colonization by more dangerous pathogens" (Mackowiak 1983: p. 83). Endothia parasitica is an opportunistic fungal pathogen of a number of trees, requiring wounds in the bark to initiate an infection (Krebs 1985). In its native Asia, it causes little damage to forest trees, but since introduction into North America, it has virtually wiped out the American chestnut tree over its whole geographic range in the Appalachian mountains. The sweep of Dutch elm disease across the United States provides a similar example involving near elimination of both urban and natural populations of the American elm.

In some cases, it is not clear whether genetic changes, environmental changes, or some combination of the two has precipitated a sudden shift in the virulence of a pathogen. The deadly AIDS virus has only recently been recognized, yet it now constitutes a major health hazard to an increasing fraction of the entire populace. It now appears that the AIDS virus was endemic in other areas of the world, but with far less dramatic effects on infected individuals. Has the AIDS virus changed genetically, has the environment offered by its hosts changed, or has some combination of genetic and environmental changes triggered its apparently sudden increase in virulence?

Other difficulties exist, beyond those of classification and the genetic and environmental plasticity of pathogenicity, if genetic engineering with "opportunistic" pathogens is exempted from close regulation and review. For example, many opportunistic pathogens, being less specialized in their exploitation of host species, are more likely to infect a wide array of hosts, with two possible consequences. First, as the number of potential hosts increases, so will the likelihood that some subset of these hosts are

hypersensitive to the opportunistic infection, requiring a more modest degree of compromise to become vulnerable to infection. Therefore, it becomes increasingly important, yet also increasingly difficult, to rule out unintended effects by opportunistic pathogens on non-target organisms. Second, by virtue of their ability to infect a wide array of host organisms, opportunistic pathogens are likely to encounter a variety of different outright pathogens, whose infections may initially compromise the host organism. Opportunistic pathogens may therefore be especially important agents in transferring genetic material to or from outright pathogens. For example, the acquisition of plasmid-encoded penicillin resistance has made treatment of the gonococcus Neisseria gonorrhoeae more difficult; the genes encoding this resistance were apparently derived from enteric bacteria, many of which can be opportunistic pathogens in the urinary tract (Roberts et al 1977).

In summary, we find the assertion that the release of genetically engineered organisms derived from opportunistic pathogens should be exempt from the detailed review recommended for outright pathogens to be untenable based on much modern knowledge about the ecology, genetics and evolution of pathogenic microorganisms. Genetic engineering involving insect and plant pests pose similar problems.

Inter-Generic and Intra-Generic Transfers

There have been several fundamentally inaccurate claims made during the debate over the possible ecological risks associated with the deliberate release of genetically engineered organisms into the environment. In particular, some proponents of unregulated deliberate release have argued that genetic engineering represents nothing more than an extension of traditional breeding practices employed by humans over many centuries. This statement is, quite simply, not true. It ignores the extraordinary advantage of the new recombinant techniques over selective breeding programs. Recombinant techniques allow specific exchanges of functional genetic sequences across taxonomic boundaries where the same genetic exchange could never be accomplished by traditional breeding methods. The authors of the Framework are to be commended for implicitly refuting this argument by acknowledging that genetically engineered organisms constructed from the DNA of species differing so greatly as to fall in different genera deserve especially close review, since the fate, ecological effects, and transmissibility of these constructs are likely to be extremely difficult to predict a priori. However, we must disagree, on several grounds, with the explicit exemption for review of those constructs that are obtained by combining the DNA of species belonging to the same genus, or by extension the same species.

Taxonomic groupings are in a seeming perpetual state of flux, with the classification of organisms into species, genera, and higher taxonomic categories constantly changing as scientists learn more about the phylogeny of all organisms. Molecular techniques ranging from protein electrophoresis through DNA sequencing and hybridization are now bringing to light new information on the genetic similarities and differences of many organisms, often radically altering our prior interpretations of evolutionary relationships. This is especially true for microorganisms, where earlier systematic studies necessarily relied on a comparatively few morphological, ecological, and biochemical traits. As noted earlier, bacterial pathogens in the genus Shigella are now known to be so similar genetically to Escherichia

coli that they can be regarded as belonging to the same genus and even the same species (Selander et al 1986). As another example, recent studies of bacteria in the genus Legionella indicate that several genetically distinct species have been confused with L. pneumophila, including some which show very low DNA sequence homology (Selander et al 1985).

The rapid and recent progress in molecular techniques for elucidating taxonomic relationships might seem to suggest that it is only a matter of time before we have correctly assigned each organism to its appropriate species, genus, and so on. Unfortunately, there are not now, nor are there ever likely to be, universally accepted standards for defining the species, the genus and each of the higher taxonomic categories. This is because these categories have no precisely defined limits, but rather represent subjective categorization of organisms which have been and continue to be subject to varying patterns and rates of evolutionary change. Even if some uniform standard could be agreed upon (e.g., based on percent sequence homology), this could only represent a categorization of relationships which in fact tend to range over a continuum from closely to distantly related. Biological taxonomy serves to organize the description and naming of intervals or degrees of genetic relatedness. In many groups of organisms taxonomic delineations are assisted by rather obvious discontinuities in relatedness and present-day gene flow, but in other groups natural gene flow or close genetic and phenotypic similarity bedevil the labors of taxonomists. Such variation in the continuity and discontinuity within the natural, biological, substratum for taxonomy and phylogeny is surely to be expected when that substratum is itself the product of unending organic evolution.

In fact, the only taxonomic category for which there is anything approaching an objective definition is that of species. According to Mayr (1970), "Species are groups of interbreeding natural populations that are reproductively isolated from other such groups." Within the confines of a species so conceived, genes follow vertical lines of descent, with interbreeding among organisms producing a constant shuffling of gene combinations through Mendelian segregation and reassortment. Mayr's definition can only apply logically to sexual organisms, and it is frequently a source of controversy even at the present time and for a variety of reasons.

The biological species definition of Mayr and others, seemingly has less relevance for many procaryotes, which include bacteria. These microorganisms typically reproduce in a clonal manner, with no or infrequent interbreeding and the resulting Mendelian recombination of genes. Moreover, what genetic recombination does occur in procaryotes is independent of the production of new individuals, and hence is termed horizontal transfer. To complicate definitions still further, much of the horizontal recombination of genes in procaryotes is mediated by semi-autonomous genetic elements, including viruses and plasmids. The range over which genetic exchange mediated by these vectors can occur is determined less by the degree of relatedness of the organisms than by the host range of the vectors. For example, the bacterial virus P1 can infect cells belonging to several different genera, although particular strains within a species may be resistant to infection by P1 (Reanney, 1976). And as noted earlier, the plasmid-encoded penicillin resistance now found in the gonococcus Neisseria gonorrhoeae was apparently derived from an enteric bacterium (Roberts et al. 1977). Furthermore, many of these vectors can exist either in an extrachromosomal state or integrated into the host chromosome.

What is the taxonomic status of genetic material isolated from a plasmid? Does this depend on whether the plasmid is integrated into the host? [In our opinion plasmids and other mobile genetic elements are best viewed as distinct organisms for taxonomic purposes.] On-going studies of DNA sequence homology in Salmonella sp. and E. coli suggest that the degree of genetic relatedness between these bacteria may actually vary depending on what genes one examines, perhaps because of recombination events in the distant past (D. Dykhuizen, pers. comm.).

Difficulties of semantics and classification aside, we believe that there are other problems with the exemption from close scrutiny of genetically engineered organisms that are constructed from species belonging to the same genus. Implicit in this exemption is the assumption that intrageneric gene transfer is sufficiently common in nature that such gene combinations could have already arisen by the natural processes of horizontal gene exchange discussed above. This is certainly not true for most plants and animals, and it is probably not generally the case for microorganisms. Although hybridization among closely related species of the same plant and animal genera does occur quite often in nature, barriers to gene flow sufficient to maintain the phenotypic distinctiveness of congeneric species is the more common situation. And while there is increasing evidence for occasional instances of horizontal gene transfer in eucaryotes (as might be mediated by their viruses), it is certainly far less common than in procaryotes (Krieber and Rose 1986). Even among bacteria, the propensity for horizontal genetic exchange varies dramatically. Genes that are found on extrachromosomal elements are more likely to be transferred than are genes that are normally found in bacterial chromosomes. The infectious spread of plasmid-borne antibiotic resistance in bacteria dramatically illustrates the potential for rapid exchange of these extrachromosomally encoded genes (Falkow 1975, Koch 1981, Hughes and Datta 1983, Levy 1985). Exchange of chromosomal genes also appears to be a rather common occurrence in some bacteria, including Neisseria gonorrhoeae (James M. Musser, pers. comm.) and Bacillus subtilis (Graham and Istock 1979). In contrast, studies of the patterns of linkage among chromosomal gene variants in Escherichia coli (Selander and Levin 1980, Caugant et al 1981, Selander and Whittam 1983), Haemophilus influenzae (Musser et al 1985), and Legionella pneumophila (Selander et al 1985) indicate that the horizontal transmission of chromosomal genes among strains in these species is quite uncommon. Thus, it is not reasonable to assume that intrageneric recombinants are invariably less novel, or more predictable, than those derived by intergeneric recombination.

Another difficulty with the exemption from rigorous review of genetically engineered recombinants that are derived from organisms belonging to the same genus concerns the often repeated argument that organisms created by genetic engineering will be less competitive than the parental types. If this argument is true, it implies that genetically engineered organisms should not be able to survive and reproduce in the environment, and thus reduces the likelihood of inadvertent ecological consequences. However, there are many reasons to question the generality of this argument, as discussed in the essay by Pimm and Levin, which we will not present here. But to the extent that this argument is valid, the presumptive fitness disadvantage of genetically engineered recombinants must be attributed to the disruption of internal coadaptation, or genetic homeostasis, owing to the introduction of foreign genes. Unfortunately, this then implies that intrageneric recombinants will be less likely to be subject to the reduction in competitive ability than intergeneric

constructs. In fact, Malmberg (1977) has demonstrated a similar effect in bacterial viruses. He showed that the fitness of recombinants was more likely to be adversely affected if the parental strains had evolved under a selective regime where recombination had not been allowed to occur. In contrast, if the parental viruses had evolved under conditions where recombination was quite frequent, then recombinants experienced no disruption of genetic homeostasis and no fitness reduction. Therefore, intrageneric recombinants can be expected to be less affected by any fitness disadvantage arising from disruption of genomic coadaptation, and hence will be more likely to persist in the environment than intergeneric recombinants, with the resulting increased ecological risks and uncertainty. In fact, this reasoning can be extrapolated down to intraspecific gene transfers, as well. Although such intraspecific transfers may, in general, be less novel, they may also be the most likely to persist in the environment. Finally, alterations in genomic structures at the species and generic level are likely to create new patterns of genetic homology and new avenues of free genetic exchange across previously refractory boundaries between species and genera. In the longer term the cumulative consequences of genetic engineering and release of modified organisms could lead to an increased blurring of many recent or even ancient phyletic lineages laid down during the evolution of life on earth.

In summary, we agree that intergeneric recombinants are likely to have a high degree of novelty, and therefore especially close attention should be paid to the possibility of unintended environmental effects. However, we also believe that in the evaluation of intrageneric, and even intraspecific, gene transfers, close attention should be paid to the potential for inadvertent persistence and spread of the recombinant organism after release, owing to the relatively low disruption of fitness engendered by such recombinations. Moreover, we believe that taxonomic classification is necessarily somewhat arbitrary, and that taxonomic relationships in and of themselves cannot provide a complete basis for the exclusion of certain genetically engineered organisms from careful review prior to their deliberate release. And with a long view to the future, we suggest the possibility that the release of genetically revised organisms, at all taxonomic levels of differentiation, has the potential to increasingly, inadvertently, and unpredictably alter the patterns of organismal differentiation brought about by the past evolution of life on earth.

Changes in Non-coding Sequences and Deletions

We take strong exception to the exemption from close scrutiny of those genetically engineered recombinants involving changes in regulatory sequences (vs coding sequences). We also disagree with the exemption that would apply to engineered organisms in which genetic material has been deleted (vs inserted).

Regulatory genetic elements control the level of production of gene products. Levels of gene product have profound effects, not only on the expression of individual characteristics, but also on the overall development, structure, function, and vigor of organisms. In the paragraphs that follow, we provide just a few examples of these effects, and their significance for assessing risks associated with the deliberate release of genetically engineered recombinant organisms.

The resistance of bacteria to the antibiotic tetracycline is commonly caused by possession a gene that encodes a protein that is believed to insert into the cell envelope and actively transport tetracycline out of the cell. Bertrand and colleagues have rigorously examined the effects of varying the level of expression of this gene on the degree of resistance. They have constructed strains of E. coli in which regulatory sequences (including promoters and operators) and structural genes (including the resistance determinant and a repressor protein) are altered; they have changed the overall level of expression by manipulating copy number of the tetracycline resistance operon; and they have examined the effects of gratuitous induction of the tetracycline resistance operon. Their results indicate that the level of tetracycline resistance (as measured by minimum inhibitory concentration) is profoundly affected by the level of gene expression. For example, changes in the promoter sequence alone can affect the level of resistance to tetracycline by greater than 50-fold (Daniels and Bertrand 1985). It has also been demonstrated that the level of expression of the tetracycline resistance determinant can profoundly influence the fitness of the organisms in the absence of tetracycline, as measured by hypersensitivity to gratuitous induction (Moyed, Nguyen, and Bertrand 1983). This hypersensitivity reflects the pleiotropic cost to the bacterium of expressing the tetracycline resistance determinant when it is not needed. Although there is, in general, an apparent trade-off between degree of resistance to tetracycline and hypersensitivity to gratuitous induction, this relationship does not invariably hold. One point mutation in the promoter for the tetracycline resistance determinant resulted in both an increased level of resistance and a reduced hypersensitivity to gratuitous induction (Daniels and Bertrand 1985). Analogous effects of modifying non-coding sequences in a recombinant organism engineered for release into the environment could result in recombinants that are not only extreme in their degree of expression of the engineered function (and hence more likely to have environmental effects), but also more persistent in the environment (so that any adverse effects would be more likely to be manifest).

In other examples, it appears that changes in the level of expression of structural genes, such as might arise by altering non-coding sequences, can have great affects upon the development, structure, and ecological functioning of organisms. The effects of gene product dosage can be readily seen in individuals that are suffering from Down's syndrome. This affliction does not result from change in the structure of any gene product, but rather it results from a change in the level of expression of certain gene products. In this case, the altered level of expression results from an extra copy of one of the chromosomes, and not as the result of a change in the DNA sequence in a non-coding region. However, the critical point remains the same: organisms may be profoundly affected by changes in the level of gene expression, as well as by changes in the structural gene products themselves.

It is also clear from a study of evolutionary and ecological relationships that major changes in structure and function may be affected by the differential scaling, or allometry, of characters (Gould 1977), much of which may be accomplished by changes in regulatory sequences that control gene dosage and timing (Bonner 1982). Two especially dramatic examples will illustrate this point. It is now known that humans and chimpanzees are very similar in their structural genes; King and Wilson (1975, p. 114) maintain that the morphological and other differences between these two species must "result chiefly from genetic changes in a few regulatory systems ..." Kingsolver and

Koehl (1985) have recently provided experimental evidence that insect wings could have arisen largely by changes in scaling, as might result from subtle changes in regulatory genes, that permitted early proto-wings to be derived from thermoregulatory structures. Because of the effects of changes in regulatory sequences on the level of gene expression, which profoundly influence the structure and functioning of an organism, we cannot support the exemption from close review of those genetically engineered organisms intended for release into the environment that are obtained solely be alterations in non-coding regulatory sequences.

We also oppose any outright exemption from careful review that might be given to projects proposing to release engineered recombinants constructed solely by the deletion of genetic material, although we do recognize the logic behind the thought that such constructs are intrinsically safe. That logic is as follows. The deletion of an existing gene function is far more likely to arise spontaneously than, say, the de novo acquisition of some new gene function by a series of mutations. Therefore, were a particular deletion to provide a fitness advantage to an organism, it is highly probable that it would already have been fixed by natural selection. Thus, it can be reasonably inferred that genetically engineered organisms created solely by the deletion of genetic material will not persist indefinitely in the environment.

Unfortunately, as discussed in the Introduction, persistence is not the sole criterion by which the safety of a recombinant can be judged. In particular, if genetically engineered organisms are released repeatedly and on a large scale, they may greatly affect the environment, perhaps adversely, even though the recombinants themselves do not persist. The proposed release of "ice-minus" bacteria, wherein a gene encoding a cell surface protein has been deleted (see Lindow 1985), is a case in point. These engineered bacteria, if applied in large environmental doses, could have a significant effect on the over-wintering survival of non-target organisms, including native plants and animals, weeds and insect pests. It has even been suggested that ice-minus cells might affect meteorological patterns if they become airborne in large numbers (Odum 1985). The likelihood and magnitude of the risks of adverse ecological effects in the particular case of the ice-minus bacteria may indeed be low, thus justifying first field tests and possibly thereafter wide-spread use. But the general point remains the same: it cannot be assumed that recombinants created solely by deletion of genetic material will invariably be without environmental risk.

In summary, we do not believe that it is valid to exempt from rigorous review those genetically engineered organisms intended for release into the environment that have been obtained by the incorporation of non-coding sequences or the deletion of existing sequences. However, we believe that these factors, taken in consideration with all other factors, might be useful in expediting the review of certain proposals once greenhouse and small field experiments with such genetic constructs prove to be repeatedly safe.

Transfer Vectors and Mobile Genetic Elements

To this point, we have not given explicit consideration to the actual mechanisms by which gene transfer is accomplished in the construction of recombinant organisms. Construction of recombinant strains will commonly involve the use of transfer vectors, including plasmids, viruses and specific

transposable elements. Plasmids and viruses will frequently contain mobile genetic elements, including transposons and insertion sequences, as well as antibiotic resistance markers of their own. In some instances, genetic transfers may be incompletely characterized, if techniques of "plasmid assisted molecular breeding" (Kellogg et al 1981) or "shotgun" techniques have been used in strain construction. In all proposed deliberate releases of genetically engineered organisms into the environment, detailed information on strain construction must be provided. In particular, it should be carefully documented that vector material, including especially antibiotic resistance factors and mobile genetic elements, has been excised from the recombinant organism proposed for release.

The careful documentation of excision of non-essential genetic material from a recombinant intended for release is important on two major accounts. First, the spread of antibiotic resistance factors is becoming an increasingly significant public health problem. There can be no justification for compounding this problem by releasing large numbers of genetically engineered microorganisms, with varying degrees of relatedness to pathogens, that contain antibiotic resistance markers used in the construction or maintenance of the recombinants.

It will be generally true that chromosomal genes which are not physically associated with vectors or mobile genetic elements will be less likely to be re-transferred in situ to native organisms than genes which are associated with vector sequences, especially transposons. This assertion is supported by the work of Selander and colleagues, cited above, demonstrating tight linkage of chromosomal variants in many bacterial species and thereby indicating a low level of recombination for these genes. However, the opposite may prove to be the case for bacterial species capable of natural transformation through direct exchange of DNA through the environment. These considerations are most important, because the potential for transfer of engineered genes greatly increases the difficulty of evaluating potential risks associated with the deliberate release of engineered recombinants.

Lenski (1987, copy enclosed) presents several scenarios wherein the transmission of engineered genes to indigenous organisms creates adverse environmental effects where none would otherwise have occurred. Lenski (1987), extending the results of Levin and Stewart (1977), also provides a general quantitative framework for evaluating the likelihood that such infectious transfer will occur. This likelihood is sensitive to (1) the scale of release of the engineered organism; (2) the fitness of the engineered organism, and hence its persistence after release; (3) the intrinsic rate of transfer of the engineered genetic material; and (4) the ecological fitness of any indigenous recombinants that arise in situ. Because (4) will be extremely difficult to determine for all possible recipients of engineered genes, and because (1) and (2) may be constrained by the intended function of the engineered organism, strong efforts should be made to insure that (3) is minimized by removing all genetic material from vectors and mobile genetic elements used in strain construction. We hasten to caution, however, that such efforts cannot absolutely preclude the horizontal transfer of engineered genes. Recombinants may contain vectors or mobile elements other than those used in the engineering process, or they may receive such elements from native organisms after their release. Such natural sequelae to release could lead to the eventual transposition of an engineered gene from a chromosome or non-transferrable

plasmid to a conjugative plasmid, greatly increasing the likelihood of inadvertent spread (see Levy 1985, Slater 1985 for analogous examples). Rather complex but necessary experimental tests are necessary to evaluate the possibility of subsequent outward spreading of recombinant gene sequences set free from the organisms which initially carried them into nature.

In summary, we believe that careful documentation of the excision of any vectors or mobile genetic elements from a recombinant released into the environment, taken together with information indicating probable safety on all other accounts, should expedite the review process. However, such excision cannot ensure the non-transferability of engineered genes, nor can transferability be the sole criterion in evaluating possible environmental risks. In the near future, steadily rising knowledge about all the exchanges and interactions among chromosomes, plasmids, viruses, retroviruses, bacteriophages, transposable elements, mutation, DNA repair, and gene expression and regulation, will be crucial to the constructing and evaluating of well characterized, genetically modified, organisms.

Agents for Biological Warfare

Although it may seem somewhat outside the scope of our charge, it cannot escape our thoughts as human beings that the genetic engineering of pathogens for deliberate release during warfare or political conflict is now possible. It is virtually certain that present-day genetic engineering techniques provide all that is necessary to create the most dangerous biological warfare agents yet devised, ones far more virulent or poisonous than known before. While we realize that much of the information on this topic is shrouded beneath concerns for national security, we believe that national and international interests could only benefit from public discussion of the general issues relevant to the engineering of biological warfare agents. This form of biotechnology is, by intent, intrinsically and universally unsafe should its products reach the environment.

Conclusions

Implicit in the Coordinated Framework for the Regulation of Biotechnology are two presumptions with which we cannot agree. First, it is presumed that outright pathogenicity, or pest status, is the only special characteristic of a recombinant organism that poses special risks to human and environmental safety. Second, it is presumed that only recombinant constructs that are highly novel, as exemplified by intergeneric transfer of structural genes, might pose environmental risks worthy of intense review. We have given above the specific reasoning behind our disagreement with these and other points contained in the Framework, and we have attempted to suggest other criteria that ought to be considered in reviewing proposals for the deliberate release of recombinant organisms. We would now like to step back and take a somewhat broader look at the difficulties inherent in drafting regulatory guidelines and procedures for products which are likely to be so varied and so novel, ones with which we have so little experience in the practical spheres of production and commerce.

The pathogen/pest and intergeneric criteria are quite understandable, in that they represent some of the deepest fears of our collective human experience. We are particularly frightened by "things" that infect us or

compete with us for our food supplies. We are also highly sensitive to "things" that are especially novel, and that we presume to be highly unpredictable as well.

But three points must be emphasized: (1) unintended pathogenicity is not the only potentially adverse environmental impact of deliberately releasing genetically engineered organisms; (2) unpredictable effects in ecological systems can arise from perturbations that are not highly novel; and (3) products or processes which are safe at one level of scale, may be unsafe when applied at larger or smaller scales.

We will try to give examples that illustrate these key points. These examples do not relate directly to the release of engineered organisms into the environment, but it is hoped that by drawing up sound regulatory guidelines now, analogous unintended consequences due to the deliberate releases of engineered organisms will not occur.

The use of pesticides in agricultural systems has had a number of unintended adverse effects. These include toxicity to farm workers, consumers, and various non-target species of animals, particularly birds. With hind-sight, we might argue that these direct effects of toxicity on vertebrates, like the direct effects of pathogenicity, could have been anticipated, but they were not anticipated. The rapidity with which pests and pathogens have escaped control by evolving resistance to pesticides and drugs is surprising and sobering, though as evolutionary biologists we might convince ourselves that it could have been predicted. But it was not predicted. Ecological and evolutionary systems are complex, and some indirect effects are extremely difficult to foresee. For example, there are well- documented examples where the use of pesticides has actually increased the abundance of pest populations, owing to the effects of the pesticides on predators and parasites of the pest species (DeBach 1974).

Even nutrients may have indirect, unpredictable and highly adverse effects upon ecological systems. The eutrophication of many lakes by run-off containing fertilizer or sewage has resulted in "blooms" by blue-green algae. These algae are often resistant to grazing pressures by zooplankton and fish, and large amounts of unconsumed algae eventually decay on the lake bottom, resulting in a build-up of toxic compounds and a depletion of oxygen, eventually resulting in death of fish and other animals and plants (Smith 1986). It has recently been hypothesized, with some supporting experimental evidence, that the harmful effects of acid-rain on coniferous forests may actually result from an unintended fertilizing effect of nitrogenous compounds in acid rain, which may cause coniferous trees to continue production of new needles when in fact the vegetation should be hardening for winter freezes. Again, these effects indicate the complexity of ecological systems and the importance of indirect effects. Toxicity/pathogenicity and the extent of genetic novelty or care in making new genetic constructs are not sufficient criteria for excluding unintended adverse effects upon the environment.

We believe that it is impossible at this time to draw up a simple set of criteria by which certain genetically engineered organisms can be safely excluded from close review prior to their release. We simply do not have appropriate experience with these systems at this time. This last remark

applies as fully to our knowledge and predictive capacities in molecular genetics as it does to knowledge and predictability in ecology.

We recognize that some individuals believe that parallels can be drawn between "families" of chemical compounds and "types" of engineered organisms, with regulatory guidelines for the former being extended to the latter. We do not share this point of view: the analogy is not direct. Regulation of chemicals in the environment has not been especially efficacious in protecting human health and environmental quality. We have far less knowledge and experience with the new "biologicals" than we have with environmentally applied chemicals. It would be audacious to assume that we now fully understand the molecular genetics of organisms, or most of the relations of genetic alterations to the ecological properties of organisms, given the intellectual challenges and rapid pace of basic discovery in both fields. Biological entities, from single organisms to ecosystems, are inherently complex; unlike chemicals, organisms are self-replicating and evolving. The capacity for self-propagation in organisms and genes could make it difficult to call back the unwise or unintended release of a genetically modified organism.

Our analyses and remarks to this point may give the impression that we are against the release of any genetically engineered organisms into the environment. This is not our view. In fact, we are enthusiastic about the prospects offered by biotechnological solutions to environmental problems ranging from increased productivity of agricultural systems, to the control of pests and pathogens, and the removal of chemical toxins from the earth's soils and waters. We further expect that many, if not most, environmental applications of biotechnology can be made into safe and productive ventures.

A number of categories of information, including those identified in the Coordinated Framework for Regulation of Biotechnology and others suggested in this and companion reports to the Office of Technology Assessment, will assist in evaluating the likelihood and acceptability of possible risks. The expectation of safety can best be met at present by a comprehensive, scientific review of all proposed releases of genetically engineered organisms into the environment. In the future, when we have sufficient experience with the effects of various types of genetically modified organisms on environmental processes it may be possible, with a very high degree of confidence, to exclude some types of applications from review, or streamline the review process. At present, the evaluation of possible risks associated with the deliberate release of genetically engineered organisms requires review on a case-by-case basis.

Literature Cited

1. Bonner, J.T. (ed.), Evolution and Development (New York: Springer-Verlag, 1982).
2. Caugant, D.A., Levin, B.R., and Selander, R.K., "Genetic Diversity and Temporal Variation in the E. coli Population of a Human Host," Genetics 98:467-490, 1981.
3. Daniels, D.W., and Bertrand, K.P., "Promoter Mutations Affecting Divergent Transcription in the Tn10 Tetracycline Resistance Determinant," Journal of Molecular Biology 184:599-610, 1985.

4. DeBach, P., Biological Control by Natural Enemies (Cambridge, England: Cambridge University, 1974).
5. Falkow, S., Infectious Multiple Drug Resistance (London: Pion, 1975).
6. Gould, S.J., Ontogeny and Phylogeny (Cambridge, MA: Harvard University Press, 1977).
7. Graham, J.B., and Istock, C.A., "Gene Exchange and Natural Selection Cause Bacillus subtilis to Evolve in Soil Culture," Science 204:637-639, 1979.
8. Hughes, V.M., and Datta N., "Conjugative Plasmids in Bacteria of the 'Pre-Antibiotic' Era," Nature 302:725-726, 1983.
9. Kellogg, S.T., Chatterjee, D.K., and Chakrabarty, A.M., "Plasmid-Assisted Molecular Breeding: New Techniques for Enhanced Biodegradation of Persistent Toxic Chemicals," Science 214:1133-1135, 1981.
10. King, M.C., and Wilson, A.C., "Evolution at Two Levels: Molecular Similarities and Biological Differences Between Humans and Chimpanzees," Science 188:107-116, 1975.
11. Kingsolver, J.G., and Koehl, M.A.R., "Aerodynamics, Thermoregulation, and the Evolution of Insect Wings: Differential Scaling and Evolutionary Change," Evolution 39:488-504, 1985.
12. Koch, A.L., "Evolution of Antibiotic Resistance Gene Function," Microbiological Reviews 45:355-378, 1981.
13. Krebs, C.J., Ecology, Third Edition (New York: Harper & Row, 1985).
14. Krieber, M., and Rose, M.R., "Molecular Aspects of the Species Barrier," Annual Review of Ecology and Systematics 17: in press, 1986.
15. Lenski, R.E., "The Infectious Spread of Engineered Genes," Application of Biotechnology: Environmental and Policy Issues, J.R. Fowle III (ed.) (in press, 1987). [Copy enclosed.]
16. Levin, B.R., and Lenski, R.E., "Bacteria and Phage: A Model System for the study of the Ecology and Co-Evolution of Hosts and Parasites," Ecology and Genetics of Host-Parasite Interactions, D. Rollinson and R.M. Anderson (eds.) (London: Academic Press, 1985).
17. Levin, B.R., and Stewart, F.M., "Probability of Establishing Chimeric Plasmids in Natural Populations of Bacteria," Science 196:218-220, 1977.
18. Levy, S.B., "Ecology of Plasmids and Unique DNA Sequences," Engineered Organisms in the Environment: Scientific Issues, H.O. Halvorson, D. Pramer, and M. Rogul (eds.) (Washington, DC: American Society for Microbiology, 1985).
19. Lindow, S.E., "Ecology of Pseudomonas syringae Relevant to the Field Use of Ice- Deletion Mutants Constructed In Vitro for Plant Frost Control," Engineered Organisms in the Environment: Scientific Issues, H.O. Halvorson, D. Pramer, and M. Rogul (eds.) (Washington, DC: American Society for Microbiology, 1985).
20. Mackowiak, P.A., "Our Microbial Associates," Natural History (4):80-87, 1983.
21. Malmberg, R.L., "The Evolution of Epistasis and the Advantage of Recombination in Populations of Bacteriophage T4," Genetics 86:607-621, 1977.
22. May, R.M., and Anderson, R.M. "Parasite-Host Coevolution," Coevolution, D. J. Futuyma and M. Slatkin (eds.) (Sunderland, MA: Sinauer Associates, 1983).
23. Mayr, E., Populations, Species, and Evolution, (Cambridge, MA: Belknap Press, 1970).
24. Moyed, H.S., Nguyen, T.T., and Bertrand, K.P., "Multicopy Tn10 tet Plasmids Confer Sensitivity to Induction of tet Gene Expression," Journal of Bacteriology 155:549-556, 1983.
25. Musser, J.M., Granoff, D.M., Pattison, P.E., and Selander, R.K., "A Population Genetic Framework for the Study of Invasive Diseases Caused by Serotype B Strains of Haemophilus influenzae," Proc. Natl. Acad. Sci. USA 82:5078-5082, 1985.

26. Odum, E.P., "Letters," Science 230:1338, 1985.
27. Reanney, D., "Extrachromosomal Elements as Possible Agents of Adaptation and Development," Bacteriological Reviews 40:552-590, 1976.
28. Roberts, M., Elwell, L.P., and Falkow, S., "Molecular Characterization of Two Beta-Lactamase-Specifying Plasmids Isolated from Neisseria gonorrhoeae," Journal of Bacteriology 131:557-563, 1977.
29. Selander, R.K., Caugant, D.A., and Whittam, T.S., "Genetic Structure and Variation in Natural Populations of Escherichia coli," Escherichia coli and Salmonella typhimurium, Cellular and Molecular Biology, J.L. Ingraham et al. (eds.) (Washington, DC: American Society for Microbiology, in press, 1986).
30. Selander, R.K., and Levin, B.R., "Genetic Diversity and Structure in Escherichia coli," Science 210:545-547, 1980.
31. Selander, R.K., McKinney, R.M., Whittam, T.S., Bibb, W.F., Brenner D.J., Nolte, F.S., and Pattison, P.E., "Genetic Structure of Populations of Legionella pneumophila," Journal of Bacteriology 163:1021-1037, 1985.
32. Selander, R.K., and Whittam, T.S., "Protein Polymorphism and the Genetic Structure of Populations," Evolution of Genes and Proteins, M. Nei and R.K. Koehn (eds.) (Sunderland, MA: Sinauer Associates, 1983).
33. Slater, J.H., "Gene Transfer in Microbial Communities," Engineered Organisms in the Environment: Scientific Issues, H.O. Halvorson, D. Pramer, and M. Rogul (eds.) (Washington, DC: American Society for Microbiology, 1985).
34. Smith, R.L., Elements of Ecology, Second Edition (New York: Harper & Row, 1986).
35. Stanier, R.Y., Doudoroff, M., and Adelberg, E.A., The Microbial World (Englewood Cliffs, NJ: Prentice-Hall, 1963).
36. Van der Plank, J.E., Disease Resistance in Plants (San Francisco: Freeman, 1968).
37. Williams Smith, H., and Huggins, M.B., "The Association of the O18, K1 and H7 Antigens and the ColV Plasmid of a Strain of Escherichia coli with its Virulence and Immunogenicity," Journal of General Microbiology 121:387-400, 1980.

February 2, 1987

Title: An ecosystems approach to potential perturbations of energy flow and nutrient cycles associated with environmental applications of genetically engineered organisms.

Authors: David C. Coleman
Department of Entomology
University of Georgia
Athens, GA 30602

Dr. Robert E. Hodson
Department of Microbiology
University of Georgia
Athens, GA 30602

Chapter for the Office of Technology Assessment, United States Congress

Biological Applications Program
Office of Technology Assessment
U.S. Congress
Washington, D.C. 20510-8025

I. Introduction

We examine the problems and opportunities for introduction of genetically engineered organisms (GEOs) - whether microbes, plants, or animals in ecosystems. We examine approaches which have been proposed, and the types of information that will be required.

Our main concern is with a functional approach: what are the sources and repositories of nutrients and how do these move in terrestrial and aquatic ecosystems? What are the processes by which nutrients move, and what are the key steps in nutrient movement at which perturbing forces might act on, or alter these processes?

We emphasize that this is a field with many unknowns, and we are covering just the high points. We note, throughout our paper, the areas where more information is needed.

II. Conceptual framework for introduction of GEOs into ecosystems and the biosphere

Much of the nutrient cycling activity is concentrated at or below the soil-surface in terrestrial systems, and at or below sediment surfaces in aquatic systems. Because there are so many species (estimated in the 1,000's for microbes alone per square yard of land surface), it is preferable (to scientists) to consider the various functional

roles played by the organisms in ecosystems. The functions of the organisms, or the kinds of biological processes they mediate, are of more interest than the identity or life history of any particular kind of organism.

There are two major categories of function in ecosystems - those devoted to synthesis, or building-up, and breakdown, or decomposition. Foremost among the synthesis processes is photosynthesis, the energy which is captured from sunlight, and transformed into organic matter by green plants. Two consequences derive from the photosynthetic process: (1) organic matter, remains of dead plant parts or plant products, is ultimately derived from photosynthesis and drives all biological processes in soil and in much of aquatic systems. (2) nutrient elements, required for life of all organisms, are the great contribution of soil and aquatic organisms (principally the decomposers) to all other forms of life.

Plant material reaches soils and sediments from a variety of tissues: dead leaves, stems, flowers, fruits, and woody materials. Additional contributions are made by dead roots, and the particles and chemical substances released by the roots during growth. We now consider the global setting for concerns about impacts on organic matter dynamics and nutrient cycling.

Global Change

Recently we have become aware, often painfully so, that humankind has developed

the potential to significantly alter the physical and chemical conditions of the environment on a planetary scale. Contrary to being only passive observers of natural changes in global temperature, atmospheric gas composition, soil and water chemistry, and forest coverage, we are active participants in influencing these large-scale processes. Most of the changes we have brought about have been unintentional results of our activities since the beginning of the industrial period and many are construed to be negative from both human and ecological perspectives.

Most of the induced changes that we know about involve humankind's manipulation of either living organisms or the remains of living organisms. These manipulations include such practices as conversion of natural ecosystems for intensive farming and burning of fossil fuels for energy. The new biotechnologies, such as the creation and release of genetically engineered microorganisms, plants and animals, are natural outgrowths of these attempts to produce more food and make available more energy for our expanding industrial population. Thus, these technologies may contribute, in ways mostly yet unknown, to our increasing rate of global environmental change.

As a percentage of international industrial output, technologies based on genetically engineered organisms are negligible and will probably remain so for the next decade. Thus, we would not expect major global environmental impact of biotechnology in the near future. However, it must be noted that, at present, we don't have reliable models

to predict even the short-term (not to mention the long term) effects of "standard" industrial processes. The recent losses in the protective ozone layer in the atmosphere above Antarctica, for instance, were not predicted by atmospheric models. With typical industrial pollutants, their concentrations always decrease as they are dispersed, and diluted from the point of origin. Therefore their impact is usually higher near, than away from the source. In contrast, GEO's may be in low concentrations in one location but can self-replicate and thrive in suitable habitats even far from the point of origin, or introduction.

Nutrient elements and nutrient cycles

Nutrient elements are the compounds needed by plants and other organisms for growth and maintenance - all metabolic activities. We will concentrate on the macroelements - namely nitrogen, phosphorus and sulfur, with carbon being the "backbone" of the organic matter.

Substantial amounts of nutrient elements enter soil and aquatic systems from the dead and decaying plant parts, but an additional source is derived from the soil's parent material. In the original rock the nutrients are present in forms unavailable to plants or most microoorganisms, but they are continually released, at a low rate, by moisture and chemical activity of the soil microorganisms (weathering).

Several of the processes mentioned above are indicated in a conceptual

diagram of Ecosystem Processes, as they are governed by controlling factors, which, over time, lead to Ecosystem Properties (Fig. 1).

Unlike other nutrient elements, nitrogen is supplied mostly from the atmosphere. As nitrogen is the element most required (= rate limiting) in many ecosystems, processes of obtaining nitrogen, via nitrogen fixation, play a key role in a number of ecosystems. Because there is much concern with enhancing nitrogen availability to crops, via engineered microbes, or microbes and plants, this is an important area of concern to ecologists and students of public policy.

The problem in nitrogen fixation is the energetic cost of "doing business" for the plant which has symbiotic bacteria, versus the plant which has no nitrogen-fixing associates, or symbionts. Nitrogen fixation occurs in nodules on the plant roots which have concentrated amounts of bacteria, which incorporate (fix) molecular nitrogen into organic compounds, which are then mineralized and made available to the plants. This process is energy intensive, requiring 12 units of high-energy phosphorus compounds to every unit of gaseous nitrogen fixed thus made available to plants.

III. Critical biological processes in ecosystems

The soil organisms that process mineral nutrients release nutrient elements as a by-product of energy acquisition for growth and reproduction. Because of the linkage between energy flow and nutrient release, the utilization of organic, or carbon,

I. CONTROLLING FACTORS

parent material | climate | vegetation | relief

Terrestrial | Marshland | Aquatic

II. PROCESSES

Energy Inputs and Transformations	GEO effects
Solar Radiation	-
Primary production	+
Decomposition	+
Nutrient Cycling	
Immobilization	+
Mineralization	+
Weathering	?
Translocation	?
Transport	
Erosion	-
Gasfluxes	+
Leaching	+

III. PROPERTIES (Development of Ecosystem)

Vegetation	+
Consumers	+
Soils and Sediments	
Base status	
Texture	
Microbial Population	+
Organic matter	+
Soil nutrients	
Phosphorus, Sulfur, Nitrogen	+
Salinity	

Fig. 1 - Ecosystem process and properties, as they are affected by GEO's. The factors and processes, working over time, lead to the development of Ecosystem properties.

compounds by the various soil organisms is of primary importance in soil biology.

Energy flow.

The dead roots, sloughed cells, and soluble organic products left in the soil by growing plants become the focus of intense and immediate biological activity. Microorganisms of many types are present in a dormant state throughout the soil; when fresh plant remains are deposited in this vicinity, the microorganisms quickly become active. They colonize the organic material and reproduce their own kind, populating the surface of the material and quickly using up the most readily available, "labile" or "soft" fractions of the material. If moisture and temperature conditions are favorable for microbial growth, there is intense competition for the labile materials. The organisms that win the competition are often those that can reproduce and colonize the material most rapidly. By simply being in place before other species can beome established, the fast colonizers make it more difficult for the slower kinds to succeed. As the labile materials are exhausted, however, the fast-growing species can no longer grow and reproduce. They reenter dormancy, and slower-growing but more specialized kinds of organisms replace them. These later waves of microbial colonizers have specialized enzymatic capabilities. They are able to break down and capture energy from more refractory organic components (that is, those with high molecular weights or resistant chemical bonds).

Microbial immobilization.

The relationship between energy flow and nutrient release is a complex one. Relatively undegraded plant residues are high in energy content and relatively low in nutrient elements. The primary decomposers require the same range of nutrient elements needed by plants, and tend to incorporate the nutrients from plant residues into their own tissues. This microbial immobilization predominates when energy sources for microorganisms in the soil are relatively more abundant than nutrient elements. The incorporation of wheat straw into agricultural soil provides a ready illustration of microbial immobilization. Plants growing in that soil may suffer deficiencies of nitrogen, and sometimes of other elements, until the readily available carbon components of the wheat straw have been used up and the nutrients previously immobilized by microorganisms are made available.

Mineralization and nutrient transformations.

Since the primary decomposers tend to immobilize nutrient elements, rather than release them, as they degrade organic material, subsequent mineralization must occur, or there would be no subsequent plant growth. Some events that kill microbial populations, such as drought and freezing temperatures, may cause lysis of cells and release of nutrients. These stresses are normal parts of the natural environment, however, and when drought or cold temperatures come about gradually, the

organisms are able to go into dormant resting states, and lose little of their nutrient supply to the environment. Under normal field conditions, it is probably predation on the primary decomposers that accounts for most nutrient release.

Nutrient Reservoirs and Fluxes in Aquatic Ecosystems

Living organisms are composed of a wide range of elements, most of which are present in only trace quantities. However, in many ecosystems, the rates of production of new biomass have been shown to be limited by availability of usable forms of carbon, nitrogen, phosphorus, and sulfur. In biomass, atoms of these elements are generally present in ratios of roughly 106:16:1:0.3, respectively. Thus, if one of these nutrients is relatively less available than the others, it will be the limiting factor in production. In aquatic environments, for instance, growth rates of algae and rooted aquatic vegetation depend, among other factors, on the concentration of the limiting nutrient.

In some ecosystems the bulk of the nutrients exists in inorganic form (carbon as carbon dioxide, nitrogen as ammonia or nitrate, phosphorus as phosphate, and sulfur as sulfate or hydrogen sulfide). In others, the nutrients are present primarily as the organic forms, i.e. as biomass or the degraded forms of biomass, such as peat and dissolved organic compounds in the water. Activities of living organisms cycle the nutrients between the organic and inorganic forms. In lakes, for instance, inorganic

C,N, P, etc. are taken up in daylight by photosynthetic organisms, such as phytoplankton and rooted aquatic plants, and incorporated into the structural polymers of the plant (cellulose and lignin) as well as into lipids, proteins, nucleic acids. In lakes and the sea, most of the organic nutrients are present as biomass or as dissolved organic compounds in the water. In wetland environments, such as many marshes and swamps, on the other hand, the non-living remains of plants (and to a lesser extent, animals) accumulate as detritus and peat resulting in the immobilization of most of the total nutrients in the ecosystems. In salt marshes, which have tidal replenishment of nutriients from the sea, these systems remain among the most productive (dry matter production per acre) known in the world.

Nutrients that are tied up as biomass and/or non-livng organic matter, are unavailable to support further growth of photosynthetic organisms and rates of primary production become limited by nutrient availability. Thus, the flow of carbon and energy through the food web of the system may be reduced. Mineralization of the stored organic matter back to the inorganic forms remobilizes the nutrients and renders them available again for uptake by organisms. The microbial populations in aquatic ecosystems mediate mineralization and remobilization through the degradation of organic matter.

A wide range of species of microorganisms can break down the simple organic components of plants and animals such as fats, proteins, nucleic acids, and sugars.

Thus, the carbon, nitrogen. and phosphorus in these compounds are rapidly recycled to the inorganic forms. Thus, it could be reasonably assumed that if one species of sugar degrader, for instance, became locally extinct, another would carry out that process. Thus the perturbation effect of loss of one species would be minimal. The same analogy holds for photosynthetic production of organic matter; in aquatic systems where one species of alga, for instance, has been removed, production by other species or groups of species often continues at similar rates as before. This brings us to an important point about rates of "processes" as measures of impact on ecosystems. Sometimes, from the human perspective, as managers of wilderness areas or agricultural lands, the quality of an environment can change markedly with no significant change in gross measures of the rates of processes. Fish production in two lakes may be the same (as lbs./year) when one produces mostly rainbow trout and the other mostly carp, but no human would say that the two lakes are therefore indistinguishable with regard to fish. In the case above of primary production by phytoplankton, we see again that although overall production may remain unchanged, the types of biomass produced may be different and not of equal value to humans who may want to keep an environment in its "natural" state or alter it for specific purposes. Overall primary production in lakes under stress, for instance, may be similar whether carried out by types of phytoplankton that result in growth of useful fish or by noxious, sometimes toxic bluegreen algae.

In contrast to processes that are carried out by a wide range of species or functional groups of species, decomposition of lignocellulose (lignin and cellulose), the structural complex that comprises 50-90% of the biomass of higher plants (plants other than algae and mosses), is degraded by only a very narrow range of microorganisms. Much of the energy entering an ecosystem in photosynthesis can be tied up and stored in the chemical bonds of this macromolecular complex. Thus, in ecosystems dominated by higher plants, the rates of production, transformation (into microbial and animal biomass) and degradation of lignocellulose to carbon dioxide or methane limit such important parameters as the productivity of the ecosystem, the organic content (and hence fertility of the soil) and even the eventual accumulation of fossil fuels.

The few species of bacteria and fungi that can degrade lignocellulose bring about the remobilization of the nutrients (Nitrogen, Phosphorus, Sulfur, etc.) tied up in plant biomass to usable inorganic forms. As lignocellulose is degraded, some parts of the molecule are incorporated into microbial biomass which in turn, is a major food source to small animals. The components of lignocellulose that are not remineralized or converted to microbial biomass accumulate as the humus in soil or as peat in wetlands, and ultimately, via later geological processes, are converted to fossil fuels, such as coal.

Considering that microbial degradation of lignocellulose is carried out by only a

few important organisms, it could be assumed that any natural or anthropogenically induced changes in the numbers, diversity, or activity of lignocellulose degraders might significantly alter the flows of carbon and energy (and, hence, the productivity) of an ecosystem. Introduction into an ecosystem, for instance, of an engineered organism that produces substances inhibitory to or toxic to lignocellulose- degrading fungi or bacteria might lead to greater rates of accumulation of organic matter in that system. In contrast, introduction of an organism that possessed the capability for enhanced rates of lignocellulose degradation, might result in net loss of soil organic compounds (humus), which are necessary for soil fertility. In this regard, genetically engineered microorganisms designed to be released into soils or water to degrade toxic organic wastes could present potential risks. Some toxic organic wastes which might be intended targets of engineered organisms are structurally similar to the naturally occurring aromatic compounds in peat and humus in sediments and soils. Thus the introduced genetically engineered organisms might also degrade these related natural compounds in addition to the intended target molecules, potentially altering the balance of organic matter accumulation and degradation. As the nutrient status of the affected ecosystems is linked to organic matter production and stability, release of lignin-degrading GEO's would be fraught with danger.

IV. Identification of the key steps at which perturbing forces might be expected to act on these processes.

The same major processes which are outlined in Figure 1 are those which might be susceptible to the actions of perturbing forces. Particularly in the area of decomposition/mineralization, we anticipate some possible problem areas, as noted in Figure 2.

Drawing on the experiences of researchers in the area of environmental effects, it is useful to note that one of the most sensitive indicators to environmental perturbation in a mixed-grass prairie in Montana was leaf litter decomposition. All plant species in the ecosystem subjected to SO_2 pollution continued to exist, but the litter decomposition rates were significantly decreased over the 36 month period of the study.

V. Identification of the most likely ways in which introduction of GEOs might have measurable impacts on ecosystem processes and cycles.

Following the lines of reasoning developed above, we anticipate the greatest system-level impacts to come from introduction of GEO;'s which might have the most marked impacts on key system-level processes, such as net primary production or decomposition and mineralization.

An example of concern would be an engineered "supermicrobe", which would

Fig. 2. Key processes which might be perturbed by GEO's.

	Organisms	Processes	Ecosystem
a)	Pathogens or anti-fungal competitors	Photosynthesis Net Primary Production	Agroecosystem (any changes in system properties?)
b)	Super-Pseudomonads, or Fungi	Decomposition, mineralization of lignocelluloses, aromatic compounds	Any ecosystem-Organic Matter Status
c)	Pesticides Waste compounds degradation	Decomposition mineralization	Any ecosystem desired, non-target target organisms

break down various complex organic compounds in coal tar products, several of which are similar to soil organic matter. If this organism could grow and function in a variety of soils and climates, it could endanger the nutrient status and possibly increase the erodibility of the soils or landscapes in which it occurred.

In contrast to the cautionary note expressed above, we also urge scientists and research planners to consider the possibilities presented by what is termed, "holistic systems engineering." In the area of agroforestry and agro-ecosystems (lower inputs, and higher management cropping) manipulations of crops, ground cover and intercropping are paying dividends to the farmers/managers.

Another option, open for further research, is one of systems-level research and development. Some examples of this are: 1) conjoint engineering of plant roots and root-inhabiting microbes which are engaged in nitrogen-fixation, or enhanced nutrient uptake (various symbiotic fungi, or mycorrhizae which are the fungus-root structure formed by their interaction). Several studies have shown mycorrhizal fungi require 4-12% of the carbon from photosynthesis which is subsequently translocated to the roots. The symbiotic bacteria (called rhizobia or actinorrhiza) require even more energy, as noted in section II, as the energy requirements are very high.

As large tracts of forests are incapable of being cheaply fertilized to enhance growth rates, there are long-range prospects of growth enhancement through bioengineering highly-desirable forest trees, such as pines and firs, to translocate more carbon to roots for use by free-living nitrogen-fixing bacteria or the symbiotic genera noted above, to alleviate the chronic shortage of nitrogen often experienced by forest trees.

Ironically, on the East Coast of North America, and in Western Europe as well,

there are well-documented examples of decreased tree-growth,which is approximately 20-30% lower than rates observed 50-70 years ago. These decreases are attributed to wet and dry deposition of acid rain (SO_2 and related products). Perhaps one of our greatest contributions, in a more global pespective, would be to decrease our existing pollution problems much more effectively.

Another area of considerable prospect is what could be termed "biochemical ecology." Researchers in Switzerland reported (Schlumbaum et al. Nature, November 1986) that certain plant roots produce large amounts of chitinase, a type of enzyme which is a useful chemical tool for the plant to use against pathogenic fungi. As fungal cell walls contain chitin, the enzyme prevents or inhibits fungal growth.

Thus development of bioengineered plants to repel pathogens and/or to encourage rhizosphere symbionts is a real, and intriguing prospect for development over the next several years.

VI. Probability estimates of perturbations occurring pursuant to the introduction of GEO's in the biosphere.

We take as our starting - point the extensive research which has been pursued on the introduction of exotic animals, plants, and microbes (via various insect or mammalian vectors (avenues of transport)) over the last 80-100 years. On average, about 1% of all introductions, whether accidental or intended, have proven successful,

and the new organism has flourished in its new milieu (Elton, 1958). This value may seem low, but is probably a conservative estimate,when compared with the physiological characteristics of GEO's which will be developed. In many cases (more than 90%, we surmise), with better survival characteristics "built in", as it were, there will be a greater likelihood of survival of an engineered microbe, plant or animal than for a natural, non-engineered one. This would be especially likely if the engineered organisms were derived from an already successful naturally occurring organism from the same habitat.

We totally reject the assertions of certain biologists that "nature has already tried all the possibilities" of bioengineering. Mechanisms of reproduction, whether asexual or sexual, are basically conservative, and have proceeded along less than 0.1% of all conceivable genetic recombinations that could be envisioned by scientists (Freifelder, 1983).

Thus, it behooves us, as scientists and informed citizens alike, to move cautiously and carefully forward. There is need for vigilance and rigor in the development of testing procedures. A graded sequence, from laboratory to growth chamber to greenhouse and only later on, to controlled field tests in high-security field sites will be a useful, viable approach to the problem of monitoring effects of bioengineered organisms on other organisms in ecosystems.

A recent publication by Dean-Ross (1986) notes the two-pronged approach

possible within the entire range of GEO's. She notes that engineered pathogens, perhaps ones to be used in combatting growth of other unwanted organisms, will need to be followed on a case-by-case basis. Other groups of non-virulent forms can be considered in terms of biochemical capabilities, with the knowledge that literally hundreds of species of microbes play an interchangeable role in ecosystem function, in such areas as cellulose decomposition, etc.

What are the probabilities that a genetically engineered organism will become established in a given ecosystem?

A much discussed model for predicting whether or not a newly engineered organism will become established in an existing ecosystem is that of the introduced or exotic species. Intuitively, we might predict that organisms designed to survive after purposeful release into the environment would have a higher probability of survival than would laboratory "crippled" microorganisms, such as those into which eucaryotic genes have been inserted, which might be released inadvertently. It is the former type of organism that best fits the exotic organism model. An organism with new properties can be expected to establish itself or disappear from an ecosystem independent of whether the new biological properties it possesses were acquired through genetic engineering or came with it naturally from a distant geographic location (Regal, 1986).

Basically, our experience indicates that most attempts at introduction fail. As

reviewed by Regal (1986), the literature indicates that a few introductions, however, have been local disasters (African bees, gypsy moths, fire ants, starlings, chestnut blight, etc), with the introduced species being established and, in some cases supplanting their pre-existing analogs. One reasonable explanation why most invasions fail has been that there are already in the system, numbers of individuals of the species of indigenous organism that "does" what the invader does. Thus only very large numbers of invaders introduced at once could establish a foothold in the system and supplant the native species. Moreover, it is assumed also that there is a degree of redundancy in most ecosystems with regard to the number of species that can carry out a given role in that system. Thus, even if the invader can supplant one indigenous species, others remain and tend to dampen the spread of the invader.

With bacterial populations, though, an additional pathway exists for introduction of new genetic material into an ecosystem besides replacement of one species with another. This pathway is termed horizontal transfer of genetic information and involves transfer of genes from one organism to another *via* transposable elements, pieces of DNA, such as plasmids that are separate from the bacterial chromosome and that can be passed from a donor bacterium to a recipient even of a different species. Transposable elements can carry genes that enable the recipient to acquire some of the metabolic functions that the donor had, such as resistance to antibiotics and toxic metals or the capability to degrade specific organic compounds. Thus, new genetic

traits can spread through whole populations of bacteria in very short periods.

It is likely that some genetic traits introduced into ecosystems with genetically engineered organisms could be passed via horizontal transfer to some of the indigenous bacteria of the system. Thus, a gene for, say, pesticide degradation, might spread to related species of soil bacteria. Then even if the introduced, engineered species did not survive in the system, the capability that it brought to the system might persist. Cases of this phenomenon have been demonstrated already in laboratory studies (Chakrabarty, 1981). On first analysis, this spread of the introduced gene might seem desirable. However, scenarios can easily be created wherein horizontal transfer would be undesirable, even disastrous. For instance, the example of humic substances breakdown in soil, or spread of resistance genes, mentioned earlier.

Another probable difference between natural invasions of exotic organisms and the introduction of genetically engineered organisms may lie in the degree of genetic difference between the indigenous organisms and the invaders. In natural introductions of exotic organisms, the differences can be expected, generally, to be multiple. Domesticated organisms such as cattle, for instance, differ in numerous characteristics from their wild ancestors. Most often, when such organisms are released into the environment, they do not establish significant populations, probably because they have lost, through many generations of selective breeding, the particular characreristics which render them "fit" for survival in the wild. Exceptions to this rule

exist,however, as some domesticated plants can become "weeds" in the wild and feral cats, dogs, etc. are well known.

In contrast to the many differences between domesticated (bred via traditional animal or plant selection methods) and wild forms of the same organism, genetically engineered organisms can be and are designed to differ from wild type organisms in as few as a single gene. Such organisms might not be sufficiently different from wild types to be significantly selected against, and thus might become established. Moreover, the engineered differences can be specifically designed to enhance survival of the altered organism. This point is important to consider in view of the assumption held in some circles that engineered organisms are somehow inherently less fit for survival in the wild than naturally occurring organisms are. To the contrary, some may be more fit for survival in particular environments and it is under such conditions that problems may arise.

VII. Recommendations to increase predictive capabilities in environmental biotechnology

It seems clear that the unknowns greatly outnumber the knowns in formulating predictive capability for assessing the potential impact of genetically engineered organisms in aquatic and terrestrial. Uncertainties remain at all levels of investigation from the molecular to the global ecosystem. Neither an exclusively reductionist nor an

exclusively holistic approach will suffice but both must be pursued simultaneously.

At the level of individual organisms, least is known about the potential establishment and effects of populations of genetically engineered microorganisms. Taxonomic protocols are much better established for plants and animals than for microbes, and questions remain even about the definitions of the terms, diversity, population, and species, for bacteria and viruses. Aside from epidemiology, little is known about rates of dispersal of microorganisms or of how similar the populations structures of soil or aquatic microorganisms are from on geographic area to another. In short, microbial ecology has developed parallel to, but independent of, the rest of ecology to a large extent.

A large part of the difficulty in studying the distribution of any microorganism, genetically engineered or not, in the environment has been methodological. Even the most sensitive classical methods (e.g. growing cells up on agar plates) fail to detect low numbers of individual species of bacteria in natural samples and the presence of solids in the samples (e.g. soil, sediments, or peat) make the tests even less sensitive. With genetically engineered organisms it will be necessary at times to follow not merely the organism that was introduced into the environment but one or a few particular genes from that organism whether the gene(s) stay in the original organism or spread to other indigenous organisms in the environment. To follow microorganisms at very low population densities or to follow individual genes will

require further development of, and application to field studies of, the new methods of molecular-based taxonomy. This involves methods such as determining nucleic acid homologies (the so-called gene probes) and automated cell sorting and counting techniques such as flow cytometry.

Previous studies with non-engineered organisms have revealed that how and when a microorganism is introduced into an environment is also important for its survival and establishment of a viable population in that environment. Thus studies with engineered microbes are needed to ascertain the importance to the spread within an ecosystem of the intact organisms themselves or the genes of interest within the organism of such environmental variables as the season of year of the introduction, the mode of introduction (e.g. dispersal by wind onto land surface vs. plowing into the soil), and the critical inoculum size (i.e. the minimum number of individuals needed for the introduction to "take").

Strong arguments can be made also for concentrating efforts on characterizing the extent to which engineered organisms alter the basic biogeochemical processes that the microorganisms carry out in aquatic and terrrestrial environments. New genes might enter an environment and alter rates of photosynthesis or biodegradation, for instance, while not visibly altering the microbial species diversity or the numbers of individuals of the dominant species. This might occur if the novel gene became incorporated into a common soil or aquatic species, changing its natural suite of

metabolic capabilities. Such an introduction could alter rates of critical processes, such as those decribed above, that control carbon and energy flow or nutrient availability within the ecosystem.

Thus, we need to develop pre-release test procedures that can spot, under controlled conditions, subtle alterations in the rates of processes such as photosynthesis, nitrogen fixation, nitrification, lignin degradation, soil organic matter transformations, sulfate reduction and methanogenesis. Such procedures will need to be worked out for use in small, laboratory bench-size enclosures (microcosms) and, subsequently in larger controlled environments, such as greenhouses or isolated aquatic enclosures that mimic, in critical ways, particular ecosystems. Such microcosms already exist and a large literature has developed that outlines the strengths and weaknesses of their use. What is needed now is research that can evaluate which types of microcosms to employ and how secure such enclosures must be for pre-release studies. Studies are needed also to determine a "standard" set of processes to follow and to determine how long a study (which will determine how sensitive the assays will be) can be and be practical considering the expense involved and the urgency of need for results in certain cases.

Following studies in enclosures, controlled-release experiments using small plots of land or small, isolated, aquatic environments, such as ponds, are needed prior to actual purposeful release of a "product" organism into an ecosystem. Early studies of

controlled release should be designed as a means of measuring ground-truth and refining the studies done in microcosms. These two types of evaluations are best designed in concert.

Some scientists have suggested that engineered plants are inherently safer, and easier to control than engineered microbes. We urge that attention be centered on the genes involved, and the opportunity for spread, as well. Thus genes for enhanced resistance to the herbicide, glyphosate, have been introduced into grain sorghum, Sorghum bicolor. Sorghum halepense, or common Johnson grass is a sibling (closely-related) species. The possibilities for genetic transfer from sorghum to Johnson grass are high, perhaps one in five, and the need to keep Johnson grass in check, rather than enhancing its herbicide resistance, is obvious.

Concerning genetically-engineered microbes, it is as yet very difficult to predict the long-term fates of bacteria to leach minerals or metallic ones, or bacteria to clean up oil spills or toxic wastes. The former, if active only at extremes of pH (very high or low acidity, for example) might be more safely managed than an oil-degrading pseudomonad, which might get spread on an ocean oil slick, thriving at a near-neutral pH.

Thus, very basic research in microbial ecology is critically needed to bring our level of understanding of natural microbial populations up to parity with our current understanding of the ecology of higher organisms. Serious efforts need to be initiated

to make terminologies used by microbial and "macrobial" (larger organism) ecologists more uniform.

At the same time ecologists, both those working with microbes and those working with higher organisms need to be formally exposed to the principles of, and techniques involved in, the genetic engineering process and the geneticists need a rudimentary knowledge of ecological theory. To some extent this process is underway now with workshops and meetings that are bringing these disciplines together. However, the funding of interdisciplinary research projects will be needed to galvanize these diverse groups into coherent, synergistic teams that can attack problems of introduction of GEO's in the environment.

Literature Cited

Chakrabarty, A. 1981. Laboratory Breeding of a bacterium for enhanced degradation of 2-4,5,T. In Genetic Engineering for Biotechnology. pp. 37-40. In Eds.: J. Crocomo, F.C.A. Tobares, and Sodrzeieski. Promocet. Sao Paulo, Brazil.

Dean-Ross, D. 1986. Release of genetically-engineered organisms: hazard assessment. ASM News 52: 572-575.

Elton, C.S. 1958. The ecology of invasions by animals and plants. Methuen, London.

Freifelder, D. 1983. Molecular Biology. Jones and Bartlett, Boston.

Regal, P.J. 1986. Models of genetically-engineered organisms and their ecological impact. pp.111-129. In: H. Mooney/Drake (eds.) Ecology of Biological Invasions of North America and Hawaii. Springer-Verlag, Heidelberg and New York.

Schlumbaum, A., F. Mauch, U. Vögeli, and T. Boller. 1986. Plant chitinases are potent inhibitors of fungal growth. Nature 324: 365-367.

ECOLOGICAL IMPACT OF GENETICALLY ENGINEERED ORGANISMS ON ECOSYSTEMS

J.R Gosz
C.N. Dahm
Department of Biology
University of New Mexico
Albuquerque, New Mexico 87131

P.W. Flanagan
Ecology Program
National Science Foundation
1800 G Street N.W.
Washington, D.C. 20550

TABLE OF CONTENTS

	Page
Introduction	1
Objectives	2
Energy Flow	3
Terrestrial Biome Patterns	3
Aquatic Biome Patterns	6
Nutrient Cycles	9
Nitrogen Cycle	9
Terrestrial Ecosystems	9
Forest Ecosystems	10
Grassland Ecosystems	11
Tundra and Boreal Ecosystems	12
Desert Ecosystems	13
Tropical Forest Ecosystems	15
Aquatic Ecosystems	16
Streams and Rivers	17
Lakes	18
Estuaries and Oceans	19
Phosphorus Cycle	20
Key Ecosystem Processes/Characteristics	23
Production (Anabolic) Processes	23
Nitrogen Limitation	23
Phosphorus Limitation	24
Mycorrhizal Production	24
Stress Tolerance	25
Growth Rate	25
Oxidation (Catabolic) Processes	25
Organic Matter Decomposition	25
Nitrification	26
Disease and Pest Control	27
Species Interactions	27
Perturbation by Genetically Engineered Organisms	27
Production (Anabolic) Processes	27
Nitrogen Limitation	27
Phosphorus Limitation	30
Mycorrhizal Production	33
Stress Tolerance and Growth Rate	34
Oxidation (Catabolic) Processes	34
Disease and Pest Control	38
Recommendations	39
Facilities Development	39
General Research Objectives	40
Risk Assessment	41
Specific Research Topics	42
References	44

INTRODUCTION

This report is being written for the Office of Technology Assessment in support of its study of "New Developments in Biotechnology". We will address the ecological implications of the environmental application of genetically engineered organisms (GEO). Our approach applies an ecosystem perspective on the potential perturbations of energy flow and nutrient cycles associated with applications of GEOs.

Discussion of potential impacts requires an acknowledgement of the underlying assumptions. Our report assumes that various industries will develop GEOs and that they will be released into the environment. Furthermore, we assume the introduced genes they contain have the potential to transfer horizontally to other organisms in natural and agricultural ecosystems.

The agricultural industry has or will develop organisms with altered genes to improve pest resistance, enhance disease control, and increase productivity. Increased productivity may result from growth promoters and/or increased access to nutrients (e.g., nitrogen, phosphorus). The chemical industry and agricultural industries will develop genetically engineered microorganisms (GEM) to remove toxic chemicals persisting in soils and waters. The mining industry and others will develop GEMs to extract subsurface metals and treat sulfur and heavy metal wastes. Medical and pharmaceutical industries will develop genetically engineered vaccines and antibiotics for control of diseases in man and animals. These GEOs will be released into the environment intentionally and/or accidentally (Flanagan 1986).

There are already many examples of GEOs which this report will refer to in subsequent pages. Many additional GEOs exist in laboratories throughout the U.S. awaiting government regulations and laws governing their release and use. Agencies are under continual and growing pressure by large companies to allow field testing and to permit environmental use. Release experiments already

have occurred in other countries (e.g. New Zealand, Argentina, England). Horizontal transfer of genes in nature is very likely. Plasmids are easily transferred as evidenced by research on genes imparting antibiotic resistance in plasmids (O'Brien et al. 1985). Genes for resistance to antibiotics inserted on the chromosome also have been shown to transfer in nature (Freter 1984). Slater (1984) provided evidence for transfer of the Rhizobium nitrogen fixing (NIF) gene. These are but several of the examples of genetic transfer of genes which have resulted in a number of bills being introduced and formation of numerous symposia, meetings, and workshops addressing the growing concern of the consequences of releasing GEOs.

Objectives:

Evaluating the ecological implications of releasing genetically engineered organisms on nutrient cycling and energy flow in ecosystems will emphasize the the following objectives:

1) Describe primary processes of energy flow through different ecosystems and components important to those processes.

2) Describe major sources and repositories of nutrients that cycle in different ecosystems and the mechanisms by which cycling takes place.

3) Identification of the key points at which perturbing forces might be expected to act on energy and nutrient cycling processes.

4)Identification of the most likely ways in which the introduction of genetically engineered organisms could perturb these processes.

5) Probability estimates of such perturbations occurring, considered against the spectrum of probable and possible environmental applications of GEOs.

6) Recommendations as to what must be done in order to increase predictive capability to improve such estimates and identification of priority areas for basic and applied research.

ENERGY FLOW

Terrestrial Biome Patterns:

Energy flow resulting from the processes of photosynthesis (carbon fixation) and respiration (carbon oxidation) is a major factor regulating element cycles. Nutrient cycling rates and nutrient availability are expected to parallel patterns of net primary production (NPP) and decomposition of organic matter for different climates. Table 1 contrasts different ecosystems for carbon storage in aboveground plant tissue, dead organic matter on the soil surface, and the major processes affecting those storage compartments (i.e. net primary production, litterfall from plants, and organic matter decomposition). These ecosystems span major climatic zones. Net primary production of aboveground plant biomass (photosynthesis minus plant respiration) is influenced by climatic factors in predictable ways ranging from low to high in tundra to tropical habitats, respectively.

Table 1. Production and decomposition in six ecosystem types.

	Tundra	Boreal Forest	Deciduous Forest	Grassland	Savannah	Tropical Forest
Mean NPP ($t\ ha^{-1}\ yr^{-1}$)	1.5	7.5	11.5	7.5	9.5	30
Mean Biomass ($t\ ha^{-1}$)	10	200	350	18	45	500
Litterfall ($t\ ha^{-1}\ yr^{-1}$)	1.5	7.5	11.5	7.5	9.5	30
Litter Accumululation ($t\ ha^{-1}$)	44	35	15	5	3	5
Decomposition ($k\ yr^{-1}$)	0.03	0.21	0.77	1.5	3.2	6.0

Rosenzweig (1968) demonstrated a good relationship between productivity and actual evaporation (AET), an integrated measure of climatic temperature and precipitation. Aboveground litterfall, the transfer of energy-containing

material from living canopy biomass to organic detritus, parallels NPP closely. decomposition rate (k yr^{-1}) is the annual fractional weight loss of detrital material. A value of 1.0 means 100% of annual litterfall decomposes in 1 year. Swift et al. (1979) reported decomposition rates increase as NPP increases (and as AET increases), but the range for decomposition values is much larger (Table 1). Tropical forests have very high decomposition rates. Complete decomposition occurs in a few months. In contrast, tundra litter may take in excess of 30 years to decompose resulting in large accumulations. This results in an inverse relationship between NPP and organic litter accumulation. Melillo and Gosz (1983) contrasted biomass, litter, and soil carbon for several forest biomes (Table 2).

Table 2. Distributions of carbon (g C m^{-2}) by biome.

	Tropics	Temperate	Boreal	Woodland Shrubland	Desert
Vegetation	20,969	16,042	10,000	6,000	1,000
Litter	147	1,208	2,000	282	50
Soil	10,400	11,800	14,500	6,900	5,600
Total	31,516	29,050	26,500	13,182	6,650

The pattern of soil carbon parallels litter accumulation, both of which are inversely related to aboveground plant biomass. The biomes which are subject to relatively more water stress (high temperatures and low precipitation) differ in some ways. Grasslands, savannahs (table 1), woodlands, shrublands, and deserts (table 2) have low vegetation aboveground biomass with respect to NPP and low litter accumulation with respect to the litterfall process. High decomposition rates in these warm environments account for the relatively low litter accumulation. In more arid areas, AET may underestimate the rate of decomposition because of behavioral adaptations of decomposer organisms that allow abiotic constraints to be circumvented

(Whitford et al. 1981). In the Chihuahuan and Sonoran deserts, activity by surface-foraging subterranean termites increased litter mass losses of two common grasses by 2 or 3 times compared to plots with termites eliminated. This is the magnitude by which the AET model of Meentemeyer (1978) underestimates decomposition rates.

Soil invertebrates play a significant role in decomposition in other environments as well. Zlotin and Khodashova (1980) contrasted first-year decomposition rates for leaf litter of several deciduous species in the presence of various soil organisms. Of the total weight loss, the larger soil fauna consistently caused the largest weight loss. Abiotic decomposition and soil microflora were responsible for the least weight loss. The larger fauna create a wide spectrum of secondary resources which are decomposed by the diverse community of microfauna, bacteria and fungi. Microfauna, bacteria, and fungi seem to influence decomposition more than macrofauna in the later stages of detritus decomposition.

There are major differences in the soil organism communities of different biomes. The amount of organic matter and climatic conditions influences microfauna and mesofauna population sizes. Deep organic soils of temperate forests maintain the highest populations of mesofauna. Trees which produce acid litter decrease earthworms, millipedes, and isopods, and increase protozoa, nematodes and Enchytraeid worms. Higher numbers of mesofauna in acid forest soils than in neutral or basic forest soils are likely and produce an inverse correlation of mesofauna populations with decomposition rates in different biomes (Swift et al. 1979).

The literature on belowground productivity, litter produced from roots, and root decomposition is sparse and primarily from forest studies. A large part of the net primary productivity is directed to the roots and fine-root growth. Total root productivity is considerably greater than 20-30% of the

tree biomass found belowground at any one point in time because of continual initiation and death of fine roots (Hermann 1977). Harris et al. (1977) reported total root production 2.8 times greater than net annual stem wood production. Agren et al. (1980) and Grier et al. (1981) estimated that 50-66% of assimilated carbon dioxide was used for fine root production. Recognition that root mass has a high turnover rate has recast thinking of both energy flow and nutrient cycling in forests; in some situations fine-root turnover (belowground litter) may be a more significant factor than aboveground litter. Vogt et al. (1982) estimated that fine roots plus mycorrhizae in a stand of Abies amabilis (Pacific silver fir) cycle some 4 times the N, 6-10 times the P and K, 2-3 times the Ca, and 3-10 times the Mg as does aboveground litterfall.

For a given species or ecosystem type, large differences also can occur in the organic detritus accumulation and decomposition rates due to site differences. Florence and Lamb (1974) reported a range of forest floor accumulations of 12-28 tons ha^{-1} for Pinus radiata (Monterey pine) stands of similar age and site quality, but differing primarily in soil type. Variation was attributed to different decomposition rates arising from differences in needle tissue quality, decomposer activity, and stand microclimate. Carey et al. (1982) reported that litter accumulation ranged from 5.9 to 59 tons ha^{-1} in 41 first-rotation Monterey pine stands. Gosz (1981) reported a range of decomposition constants for conifer ecosystems from 0.008 to 0.69. Comparisons of a given species or community type on different sites are scarce, although they suggest that the ranges for litter accumulation and decomposition rates may be similar in magnitude to the range found over different biomes (Table 1).

Aquatic Biome Patterns:

The range of mean NPP values for aquatic ecosystems is similar to

terrestrial ecosystems (Table 1). For example, the lowest production rates are 1.3 tons ha^{-1} yr^{-1} for open ocean regions, comparable to the 1.5 tons ha^{-1} yr^{-1} rate for the tundra. Highest rates of mean NPP in aquatic ecosystems are 20 tons ha^{-1} yr^{-1} for swamps and marshes and 25 tons ha^{-1} yr^{-1} for oceanic algal beds and reefs. These values compare well to the mean NPP of 30 tons ha^{-1} yr^{-1} for tropical forests. Other rates of mean NPP in aquatic ecosystems include 2.5, 3.6, 5.0, and 15.0 tons ha^{-1} yr^{-1} for 1) lakes and streams, 2) continental shelves, 3) upwelling oceanic zones, and 4) estuaries, respectively (whittaker 1975). Decomposition rates in thes environments are quite variable with temperature and chemical composition of the waters important regulating factors.

Energy in aquatic ecosystems derives both from external inputs from terrestrial environments and in situ production (Cummins 1974, Wetzel 1983). Small streams in forested environments depend on external sources of organic carbon for energy (Fisher and Likens 1973). As the stream grows in size, more light reaches the channel and aquatic photosynthesis increases in importance (Vannote et al. 1980). Algae and rooted vascular plants supplant external inputs as the major source of energy. Larger rivers are once again more dependent on imported energy sources as turbidity and depth restrict primary production. A continuum of sources of energy and subsequent biological responses are predicted to occur from headwaters to river mouth (Vannote et al. 1980).

Lakes too derive energy internally and externally (Wetzel 1983). Major internal sources include phytoplankton, attached algae, and macrophytes. External sources are largely dissolved organic carbon (DOC) with lesser amounts of particulate material. Overall productivity is a major factor in determining which energy sources predominate. For example, Lawrence Lake, Michigan, a productive temperate hard-water lake, had 41.3% of its annual carbon budget

come from macrophytes and 43.3% from the various algal sources. In comparison, Mirror Lake, New Hampshire, an oligotrophic mountain lake, had only 3.1% of its annual carbon inputs from macrophytes and 73% from algal sources. The rest of the energy to the lakes came from terrestrial ecosystems, primarily as DOC.

Estuarine and coastal oceanic environments also may receive much of their energy supply from external sources (e.g. Naiman and Sibert 1979, Button 1984), but, in general, most of the energy is photosynthesized locally (Haines 1977, Simensted and Wissmar 1985). Energy flow is primarily through detrital processing. Salt marshes and tidal mudflats process much of this energy through anaerobic pathways (Howes et al. 1984, Howarth and Hobbie 1982, Valiela et al. 1982). Belowground production of salt marsh plants can often exceed above ground production (Howes et al. 1985). The above- and belowground production makes these ecosystems extremely productive, rivaling tropical rain forests.

Open ocean environments must efficiently cycle energy internally as limited external supplements are made available. Riley (1963) has concluded that a flexible system of reversible reactions exists that allows organisms to draw upon the pool of organic carbon and also replenish it. Most organic input into the system occurs in the photic zone through phytoplankton. The phytoplankton then contribute energy and biomass to both grazers and decomposers. Organic material is substantially recycled in the photic zone with limited losses of energy reaching the aphotic zone and benthos. A large proportion of the organic energy is remineralized by the heterotrophic populations, releasing much of this energy for reuse by the photosynthetic community (Parsons and Takahashi 1973). A carefully balanced cycling system maintains production and cycling in these environments. Although production rates are generally low, the vast extent of the oceanic environment produces approximately 25×10^9 metric tons per year compared with 50×10^9 for all land based primary producers (Bolin 1970, Woodwell 1978).

NUTRIENT CYCLES

This section discusses sources, repositories, and cycling characteristics of important nutrients. Many levels of resolution are possible in descriptions of nutrient cycling, ranging from annual budgets on a global scale (Delwiche 1977, Soderlund and Svensson 1976) to detailed process studies in one environment. Our approach will be to identify important sources or processes and contrast different nutrients and ecosystems. More detailed description will be developed for those processes most likely to be affected by GEOs.

NITROGEN CYCLE

Terrestrial Ecosystems:

Nitrogen is the nutrient most often cited as limiting production in terrestrial ecosystems. The literature on nitrogen is voluminous and only a brief survey is presented.

The nitrogen cycle is the most complex nutrient cycle because the element can exist in many oxidation states which confer varied chemical characteristics. It is subject to biological and chemical immobilization, leaching and gaseous loss. Biological mineralization is dependent on a complex interplay of temperature, aeration, water, and type and amount of organic matter. Great diversity exists within and between the nitrogen cycles of different ecosystems.

The main storehouse of nitrogen is the atmosphere, but the transfer rate of nitrogen from the atmosphere to the biosphere is low. Plants obtain the majority of their nitrogen from the soil by absorption through roots and mycorrhizae even though the fraction of soil nitrogen in a readily available form at any one time is rarely more than 1% (Scarsbrook 1963). The element is not limiting, but rather fixed forms of the element are. Genetic engineering, by manipulating the nitrogen-fixing gene complex (NIF), may add substantially to the availability of nitrogen.

Forest Ecosystems: While coniferous and deciduous forests can attain similar NPP and plant biomass, there are marked differences in storage and cycling of nitrogen.

Table 3. General characteristics of conifer and deciduous hardwood ecosystems (from Rodin & Bazilevich 1967, Larcher 1975).

	Conifer	Relative Difference	Deciduous Hardwood
Plant Biomass (t ha^{-1})	50-350	=	150-300
Biomass N (kg ha^{-1})	300-1900	=	250-1600
Foliage Biomass (t ha^{-1})	5-20	>	1-5
Nitrogen (%)	1-5	<	1.5-3.0
Forest Floor (t ha^{-1})	10-70	>	5-60
Net Primary Prod. (t ha^{-1})	7-20	=	7-20
Total Litterfall (t ha^{-1})	2-7	>	5-7
Leaf Litterfall (t ha^{-1})	0.3-6	>	1-5
Leaf Litter N (%)	0.4-1.3	<	0.5-1.8
Litterfall N (kg ha^{-1} yr^{-1})	10-90	<	45-90
N Uptake (kg ha^{-1} yr^{-1})	30-75	<	50-100
N:Ca of Uptake	>1	>	<1
N Mineralization (kg ha^{-1} yr^{-1})	50-100	<	100-300

Conifer ecosystems have lower rates of nitrogen cycling resulting from lower nitrogen fixation, mineralization, and uptake. Acid, nutrient-poor conifer litter is difficult to decompose and accumulates on the forest floor.

The internal redistribution (i.e. retranslocation) of nitrogen from leaves or needles before they senesce and fall as litter is a major factor in their low N%. Other aspects of tissue quality also change with nitrogen availability. For example, production of polyphenols is markedly affected by alteration of environmental factors and nitrogen availability (Davies et al. 1964, Coley et al. 1986), and has been linked with plant ability to resist stress (Rowan 1970). A higher phenolic content of plant litter from either deciduous or conifer species decreases its rate of decomposition by: a) reacting with plant proteins to produce resistant complexes; b) coating or encrusting decomposable plant substances with resistant polyphenol complexes; c) complexing of plant cellulose and hemicellulose with polyphenols; and d)

reaction with microbial enzymes (Benoit et al. 1968, Benoit and Starkey 1968a,b). This will be an important area in our consideration of the influence of certain types of GEOs. A large range of variation in litter decomposition and forest floor accumulation rates exist. These two rates may be very sensitive to changes from GEO introduction.

Grassland Ecosystems: The biogeochemistry of nitrogen in grasslands is similar to that in forest ecosystems; however, the distribution of many components and the rates of many processes are vastly different. Notable are the annual nature of most of the aboveground biomass, the dominance of belowground biomass and its influence on pathways and transformations in nitrogen cycling, and the important role of larger herbivores (Woodmansee 1981). Table 4 lists representative ranges for nitrogen in various components of grasslands. More than 90%, often more than 95%, of the nitrogen is in soil organic matter. Mineral nitrogen is less than 0.5% of the total nitrogen.

Table 4. Representative ranges for nitrogen contained in various components of grasslands (Woodmansee et al. 1981).

Component	Range ($g\ N\ m^{-2}$)
Tops (live)	1-10
Tops (dead)	0-2
Litter	<1-9
Roots (live)	2-10
Roots (dead)	2-20
Microorganisms	2-15
Soil Organic Matter	90-1600
Invertebrates	<0.01-0.3
Large Vertebrates	0-3

Plants and microorganisms both take up inorganic nitrogen during periods of rapid plant growth. Uptake capabilities of microorganisms and plants in unfertilized grasslands generally exceed the mineralization rates. Thus, essentially all mineral nitrogen is immobilized in living organisms. Plants and microorganisms are in intense competition often limiting their growth.

Nitrogen-fixing legumes are not a dominant component of the vegetation in

most perennial grasslands, frequently accounting for less than 5% of the primary productivity. Annual nitrogen fixation rates are generally less than 1 kg ha^{-1} (Paul 1976). In annual grasslands, nitrogen fixation by legumes can be large some years (>50 kg ha^{-1}), but virtually non-existent in others (Jones and Woodmansee 1979).

Denitrification is not common in grassland soils. Low nitrate content and generally oxygenated conditions inhibit denitrification. Mineralization of organic matter produces ammonia which is largely taken up by plants during the growing season. During periods when the vegetation is dormant, nitrification can be an important process.

Tundra and Boreal Ecosystems: Severe climatic conditions make these environments distinct. Permafrost, organic matter accumulation in wet areas, and evaporative conditions in dry areas place strong conservative geochemical controls on nutrient cycling. Tundra soils may retain more than twice as much N as temperate grassland, bog and desert soils (Van Cleve and Alexander 1981). The C/N ratios are generally lower for tundra than for forest plants. Low C/N ratio plant material usually results in more extensive mineralization and greater loss of mineralized nitrogen; however, low temperature restricts organic matter mineralization. Rosswall and Granhall (1980) estimated nitrogen turnover in soil organic matter, microbial biomass and soluble and exchangeable soil inorganic nitrogen to be about 3, 60, and 33 times more rapid in a temperate mixed forest compared with the tundra.

The influence of cold is evident in reduced levels of primary production and soil organic matter mineralization and in smaller intra-system fluxes of nitrogen. Annual dry matter production may decline by more than 20-fold from tropical forest to arctic tundra and an order of magnitude from boreal forest (9000 kg ha^{-1} yr^{-1}) to arctic tundra (1000 kg ha^{-1} yr^{-1}). The reduction in productivity is reflected in smaller N uptake and reduced return

of N in litterfall for arctic ecosystems. Average nitrogen return in litterfall declines from 80-90 kg ha^{-1} yr^{-1} at latitude 50N to about 5 kg ha^{-1} yr^{-1} at latitude 75N. Net ecosystem N mineralization declines from about 60 to 70 kg ha^{-1} yr^{-1} in temperate deciduous forests and desert ecosystems to approximately 10 to 20 kg ha^{-1} yr^{-1} in low and high arctic ecosystems.

A number of important mechanisms result in nutrient conservation and minimal loss in these ecosystems: a) permafrost retention by preventing deep leaching; b) retention in slowly decaying organic remains; c) high cation-exchange capacity; d) reduced external nutrient supplies; e) reduced autotrophic nitrification; and f) slowed rate of organic matter mineralization. Other mechanisms are associated with nutrient conservation that help to maintain ecosystem function. They include the ability of the microbial population to function at 0 C or below, unique morphological and physiological adaptations of high root to top biomass and the ability to initiate growth and nutrient uptake under cold air and soil temperature conditions.

Desert Ecosystems: Moisture in desert biomes is relatively unavailable during the greater portion of each year. Actual evapotranspiration greatly exceeds precipitation. Also, moisture inputs are highly erratic (Bell 1979). Noy-Meir (1973,1974) proposed that arriving 'pulses' of precipitation drive processes such as production, consumption, and decomposition. Deserts also may have the greatest spatial variation in soil characteristics of any life-zone. Numerous publications have described the 'islands of fertility' that exist around desert trees and shrubs (see Crawford and Gosz 1982). Plants differ in their contribution to such 'islands', different species absorb various minerals in different quantities and from different soil horizons, all of which markedly influence nutrient cycling characteristics.

In addition to water availability, minimal amounts of vital elements can

be secondary controlling factors of desert productivity. This was recognized after productivity studies demonstrated that plants do not respond as expected to wetter-than-average years or irrigation. While water remains the dominant factor, limiting nutrients can influence the efficiency of water-use by plants.

Nitrogen fixation is important in deserts. Biological fixation is accomplished by legume-*Rhizobium* symbiosis, by associative symbiosis in the rhizosphere, and by photoautotrophic cyanobacteria (blue-green algae), either free-living or in association with lichens (Skujins 1981). Legumes are common in desert communities and are potentially the most important fixers of nitrogen. Nitrogen fixation in the rhizosphere has been demonstrated and numerous plant families in arid ecosystems probably have the potential for this function (Skujins 1981), though limited to brief periods of favorable moisture and temperature. Fixation by cyanobacteria can reach 30 mg N m^{-2} yr^{-1} (Rychert and Skujins 1974). Fixation does not occur except in relatively moist soil; however, it can begin less than an hour after moisture becomes available. In the Sonoran Desert, 3 to 4 g N ha^{-1} may be fixed following a fall of rain (MacGregor and Johnson 1971). Dew also may promote cyanobacterial N fixation (Rychert et al. 1978).

The low precipitation of arid regions makes leaching losses practically non-existent; however, the torrential nature of much of the precipitation that does fall can result in erosional losses of organic nitrogen in surface litter. Nitrogen loss by biological denitrification is considerable in some desert ecosystems. Denitrification rates in deserts are highly variable, but do not increase with increasing moisture, temperature, organic carbon, or organic nitrogen.

It is more difficult to construct a nitrogen budget for desert ecosystems because the magnitude and ranges of inputs and outputs are much greater. A conservative estimate of the flux of nitrogen into and out of a 'typical'

desert ecosystem may be in excess of 30 kg ha^{-1} yr^{-1}. This value is much larger than that of most other ecosystems, even greater than many tropical ecosystems (Likens et al. 1977). This relatively high flux does not cause large accumulations of nitrogen in desert ecosystems; in fact, the total size of the biotic and abiotic pools in deserts is commonly lower than in more mesic biomes (Table 2).

Although productivity is low in deserts, rates of nitrogen uptake by plants appear high. Desert shrubs contain about 3% nitrogen in their leaves. In the Mojave Desert the leaves of many species exceed 4% N (Skujins 1981, Wallace et al. 1978). These values are higher than in most other ecosystems, and are not characteristic of an ecosystem that is 'nitrogen deficient'.

Tropical Forest Ecosystems: Nitrogen cycling patterns in tropical forests have a number of distinct differences (Table 5). One major factor is the low amounts of nitrogen in the soil relative to the living plants. Nitrogen return to the soil via litterfall is a dominant pathway in tropical forests. There is a continual fall of litter and the nitrogen content is high. A large transfer of nitrogen from the canopy to the soil also occurs with throughfall (canopy wash). These large transfers from the canopy contribute to rapid cycling of nitrogen; a very different pattern from the conservative and slow dynamics typical of temperate and boreal forests.

Root production and turnover are also massive. Herrera and Jordan (1981) estimated a return of 225 kg N ha^{-1} yr^{-1} to soil from dead fine roots. In tropical forests with very even climatic conditions, the processes of root die-off, decomposition, mineralization and possibly uptake by active roots are expected to be continuous.

Biological fixation of nitrogen ranges as high as 200 kg N ha^{-1} yr^{-1}, and helps renew nitrogen capital losses from runoff. Losses of nitrogen in drainage are high compared to other ecosystems; however, they are relatively

low compared to the internal fluxes of nitrogen and the inputs from biological fixation and precipitation.

Although the general pattern of nitrogen cycling in tropical forests is one of massive quantities of nitrogen moving rapidly, there is considerable variability among different tropical forests. The data of Table 5 demonstrate some of that variability (e.g. litterfall N) which can occur because of soils of varying fertility.

Table 5. Comparison of nitrogen cycling in different tropical forest ecosystems (Herrera and Jordan 1981).

	El Verde Puerto Rico	Banco Valley Ivory Coast	Kade Ghana	Manaus Brazil	Rio Negro Venezuela
Standing stores (kg ha^{-1})					
Aboveground veg.	1200	1400	1800		336
Belowground veg.	30		210		834
Total vegetation	1230		2010	5360	1170
Litter	130		35	280	132
Soil		5800	4600	3012	785
Flows (kg ha^{-1} yr^{-1})					
Rainfall input	10	20	15	6	21
Throughfall	70	60	12		9
Retranslocation					9
Fine litterfall		147	200	89	24
Woody litterfall		9	40	15	4
Total litterfall	160	156	240	104	28
Root turnover					250?
Biological N fixation	88				35-200?
Stream output	30			5	13

Aquatic Ecosystems:

Aquatic ecosystems range in scale from the smallest streams and ponds to the oceans of the world. In all cases, nutrients and their cycling play key roles in the productivity and energy flow within these environments. These nutrient cycles and energy cycles are strongly interlinked. Perturbation of one component often has reverberations echoing throughout many of the other cycles. Although nutrient cycles are often considered on an element by element basis, the strongly interactive and interdependent nature of these processes should not be overlooked (Likens 1981). The following discussion considers primarily

nitrogen and phosphorus cycling, but their links to carbon and energy flow and the other key elemental cycles should be remembered.

Streams and Rivers: Stream ecosystems accumulate and transport nearly all the nitrogen loss from a watershed (e.g. Likens et al. 1977, Triska et al. 1984). Disturbance within the watershed such as fire, herbiciding, clearcutting, agriculture, or erosion increases the supply of nitrogen to the stream (Likens et al. 1970, Sollins et al. 1981, Sollins and McCorison 1981). Nitrogen inputs are in the form of particulate or dissolved organic nitrogen or inorganic nitrogen, usually in the form of nitrate or ammonia. The form of the incoming nitrogen varies greatly from watershed to watershed. For example, Gosz (1978) and Triska et al. (1984) showed that dissolved organic nitrogen (DON) dominated outputs of nitrogen to coniferous forest streams in Oregon and New Mexico, which contrasts with Bear Brook, New Hampshire, where 87% of dissolved nitrogen transport was nitrate and ammonia and only 13% was DON (Meyer et al. 1981). Agricultural watersheds often have significantly higher inputs of both inorganic and organic nitrogen. These various sources of nitrogen, once input into the stream, are then cycled within the water, sediments, and at the sediment/water interface.

Cycling of nitrogen within streams and rivers includes a complex set of processes and transformations involving diverse biotic communities with considerable spatial and temporal variability. These processes include leaching and mineralization of the particulate organic nitrogen (PON), DON uptake and mineralization, nitrogen fixation, nitrification, denitrification, ammonia assimilation, and nitrate reduction. No single stream study has examined all these processes within one system, but individual processes have been measured in separate studies. For example, Hill (1983) showed that denitrification and nitrate reduction within the sediment of a stream and river draining agricultural land was a major pathway for nitrogen removal during summer

months. Grimm and Fisher (1986), Triska et al. (1983) and Gregory (1980) have demonstrated that benthic algal uptake of nitrate limits algal production in some streams when light levels are adequate. Aumen et al. (1983) showed that nitrogen concentration and chemical form influence mineralization of lignocellulose in streams. Nitrogen fixation, either heterotrophic or autotrophic, also adds additional nitrogen to the stream economy (Horne and Carmiggelt 1975, Triska el al. 1984, Ward et al. 1985). Nitrification converts ammonia to nitrate and often is centered with nitrifying populations at the sediment/water interface (Chatarpaul et al. 1980). Unidirectional flow of streams and rivers and the interplay of hydrology and geomorphology create the template where the various nitrogen cycling processes occur.

Lakes: Lake ecosystems cycle nitrogen that derives from direct precipitation onto the lake, nitrogen fixation in the waters and sediments, and surface and groundwater inputs (Wetzel 1983). Alterations in the rate of supply of any of these sources affect lake productivity. Goldman (1981) described two decades of change in Lake Tahoe as nitrogen inputs from surface and groundwater sources increased. Water clarity gradually decreased and phytoplankton photosynthetic activity increased. Goldman and Horne (1983) suggested that in highly productive lakes, nitrogen fixation plays a major role while nitrogen fixation is a minor contributor to nitrogen inputs in low productivity lakes. Nitrate is assimilated and aminated into organic nitrogen within algae or higher plants, or can be converted to N_2 through denitrification. Denitrification is favored in anoxic environments and therefore is found in the sediments of most lakes and the bottom waters of productive lakes during periods of stratification (Chen et al. 1972, Dahm et al. 1983). Additional losses of nitrogen from lakes other than denitrification include effluent outflow and permanent sedimentation of inorganic or organic nitrogen-compounds. The sediments, shallow littoral regions, and open waters of the lake contribute differentially to various

processes within the nitrogen cycle in a lake and determine the chemical form and concentration of nitrogen in the ecosystem. This cycling of nitrogen in turn often affects lake productivity and the structure of the biota (Wetzel 1983).

Estuary and Ocean: Coastal and estuarine cycling of nitrogen is centered in the sediment and at the sediment/water interface (Sorensen 1983). Since the concentration of organic matter often is high, denitrification is an important process in these environments and this process is largely controlled by the supply of nitrate and nitrite rather than organic materials (Koike et al 1983). Billen (1982) summarized available data for coastal shoal-water denitrification rates at a median value of about 1.0 mmol m^{-2} d^{-1}. Large quantities of nitrogen are lost from these ecosystems as N_2. In addition, nitrate inputs from the freshwater or marine inputs, and nitrification in the surface sediments and at the sediment-water interface become critical sources of nitrate to sustain the denitrification activity. Koike et al. (1983) and Jenkins and Kemp (1984) concluded that interaction between nitrifying and denitrifying bacteria is one of the basic characteristics of these ecosystems. Their measurements of nitrification rates in coastal and estuarine studies support the concept that in situ nitrification provides much of the nitrate for the denitrifying populations (Nishio et al. 1983, Koike et al. 1983, Jenkins and Kemp, 1984). A rich source of ammonia is also needed for nitrification to be maintained at high rates in these environments. One ultimate source for ammonia could be nitrogen-fixation. Teal et al. (1979) demonstrated the importance of nitrogen fixation to the nitrogen economy of a saltmarsh. Capone and Carpenter (1982) summarized data on nitrogen-fixation in estuarine and seagrass communities. The range of nitrogen-fixation rates was .02 to 1.0 mmole m^{-2} d^{-1}, similar to the range of measured denitrification rates. Nitrogen cycling in estuarine and coastal ecosystems is a dynamic process involving both

plant and microbial populations. A good summary of a nitrogen budget for a salt marsh ecosystem has been published by Valiela and Teal (1979).

Cycling of nitrogen in marine environments has been implicated to limit net primary production in the ocean by a number of investigators (e.g. Ryther and Dunstan 1971, Thomas 1970a, b), although this remains controversial (Smith 1984). Utilization and cycling of inorganic and organic sources of nitrogen involve the phytoplankton and bacterial populations in marine systems. Much of this activity in oceanic environments is centered in the picoplankton (<1 um), nannoplankton (<10 um), and the heterotrophic bacteria (e.g. Wheeler and Kirchman 1986, Probyn and Painting 1985). Small size and efficient uptake of limited resources focus much of the nitrogen cycling into these organisms. itrogen-fixation rates in the ocean are presently being debated. Martinez et al. (1983) presented evidence that past experimental procedures seriously underestimate nitrogen-fixation. Zooplankton also play an important role in the release and cycling of nitrogen in marine ecosystems (e.g. Jackson 1980). Zooplankton feeding releases nutrients for phytoplankton and bacteria (Gardner and Scavia 1981). The limited supply of nitrogen in the photic zone of most marine ecosystems helps to promote efficient recycling of this resource. Small increases in available nutrients to the planktonic population can have major affects on ecosystem productivity as seen in upwelling regions of the ocean (e.g. Ryther 1969).

PHOSPHORUS CYCLE:

This cycle will be discussed briefly in terms of its similarities and dissimilarities to the nitrogen cycle. The importance of phosphorus is due to its central role in metabolic reactions, its relative scarcity among the light elements and its immobility or uneven distribution within various ecosystems. A close link between nitrogen and phosphorus cycling has been shown for aquatic ecosystems (Schindler 1977), but it has not been generally recognized for

terrestrial ecosystems.

The ratio of nitrogen to phosphorus in soil organic matter varies with depth in soil profiles as well as between soils. Walker and Adams (1958) and Westin and Buntley (1967) reported ratios of total soil nitrogen to organic phosphorus ranging from 2 to 11.1, with organic matter at lower depths having generally lower ratios. A major factor in the difference in nitrogen:phosphorus ratios is the source of each. Phosphorus is liberated into the soil environment by the weathering of apatite rocks. Because phosphate tends to precipitate to form materials of low solubility, sorb onto surfaces, and form complexes with metal ions, much of the phosphate released by weathering is immobilized (Van Wazer 1973). As a result, free phosphate often is present in only trace quantities and little is leached into fresh waters.

Melillo and Gosz (1983) reported significant differences in use and retranslocation of phosphorus in comparison with nitrogen. They concluded that: 1) element ratios in green foliage and leaf litter vary among sites because of different supplies of individual nutrients at the sites; 2) the withdrawal or translocation of nutrients from leaves prior to abscission is an important nutrient conservation mechanism; 3) nutrient use efficiency of one nutrient often is independent of nutrient use efficiencies of other nutrients (e.g. efficient use of nitrogen in an ecosystem does not necessarily imply efficient use of phosphorus in that system); 4) the efficiency of nutrient use is strongly related to the quantity of the nutrient cycling within the ecosystem. The quantity of a nutrient in litterfall is a measure of the nutrient cycling rate.

Melillo and Gosz (1983) also discussed phosphorus in a role of being the 'master element'. Phosphorus exerts control over nitrogen accumulation by influencing nitrogen fixation. An ample supply of phosphorus and scarcity of nitrogen are necessary for high rates of nitrogen fixation. In the very early

stages of soil formation on parent material devoid of nitrogen, nitrogen fixation is an important process. With high phosphorus availability and increasing nitrogen availability, plant production increases and organic matter begins to accumulate in the soil. In the middle stages of soil formation, losses of P are equalled by P input from weathering, the N:P ratio increases and non-N-fixers compete successfully for the N and P being mineralized from soil organic matter. The amounts of available N and P are in the optimum range for maximum plant production and organic matter continues to accumulate in the soil. Late in soil development, soil organic matter begins to decline. This occurs when apatite has disappeared or fallen to such a low value that the rate of release of P by dissolution of apatite is less than the loss of P from the system either by leaching, conversion to unavailable forms, or both. Further development of the ecosystem in climates where leaching occurs causes additional loss of P and leads to declining levels of unavailable inorganic P and organically bound P as well as organically bound C and N. Only fertilization with phosphorus can then increase system productivity (e.g. New Zealand, Australia).

The net organic carbon production of freshwater aquatic ecosystems is often limited by phosphorus availability. Vollenweider (1976) has developed phosphorus loading models to explain eutrophication in lakes. Edmondson (1970) and Edmondson and Lehman (1981) described the critical role of phosphorus in the eutrophication and subsequent recovery of Lake Washington in Seattle. Wetzel (1983) states that, in general, phosphorus is least abundant and commonly the first element to limit biological productivity in streams and lakes.

The whole-lake nutrient enrichment experiments of Schindler (1971, 1974, 1977) are particularly elegant examples of the potential role of phosphorus on aquatic ecosystems. Fertilization of one basin of Lake 226 in Ontario, Canada

with sucrose and nitrate left that basin near pre-fertilization conditions while the second basin which received sucrose, nitrate and phosphate became highly eutrophic and was covered by a dense algal bloom. Subsequent recovery of the basin which received phosphate was very rapid when phosphate additions ceased. Clearly, phosphorus can be a key nutrient in freshwater primary production. Vallentyne (1970) summarized the topic well by describing lakes with excess phosphorus inputs as "algal bowls".

The question of the role of phosphorus in limiting organic production in marine environments is controversial. Most marine geochemists conclude that phosphorus availability limits net organic production in the sea (e.g. Redfield 1958, Lerman et al. 1975, Meybeck 1982, Broecker and Peng 1982). Marine biologists believe that nitrogen is the limiting factor to algal growth (e.g. Ryther and Dunstan 1971, Thomas 1970a, b). The root of the controversy involves the ability of marine organisms to adjust available nitrogen supplies through nitrogen fixation (Smith 1984). Martinez et al. (1983) have argued that oceanic nitrogen fixation has been underestimated due to experimental artifacts of the incubation procedures. Any condition that enhances nitrogen fixation rates will place additional demand on phosphorus availability. Net marine ecosystem production of organic matter might also be attributable to phosphorus cycling.

KEY ECOSYSTEM PROCESSES/CHARACTERISTICS

This section will address our assessment of key system processes involved in nutrient cycling and energy flow, centering on those most likely to be significantly affected by anticipated genetic engineering. We have divided the discussion into: Production (Anabolic) Processes, Oxidation (Catabolic) Processes, and Disease and Pest Control.

A. Production (Anabolic) Processes:

Nitrogen Limitation: Adding nitrogen (e.g. increased N fixation) should result in a wide range of effects from direct, immediate increases of

productivity (e.g. mesic grasslands and deciduous forests) to little productivity response because the additional nitrogen will be largely immobilized in recalcitrant material accumulating slowly through time (e.g. boreal forests, tundra, bogs, temperate mires). Extensive experience with nitrogen fertilization documents this range of responses. Major differences in response and consequences are likely to occur depending on whether the additional N fixation is carried out by free-living microbes, symbiotic associations, or directly by plants (i.e. with NIF gene complex).

Phosphorus Limitation. Phosphorus deficiencies also are well documented and sometimes are related to nitrogen cycling (e.g. through N fixation and/or denitrification), especially for freshwater ecosystems. Phosphorus availability is either microbially-mediated directly or indirectly through production of extracellular phosphatases held in the organic-soil matrix (soil enzymes), or released into solution. An increase or decrease in phosphorus mineralization from primary minerals or from detrital organic matter can cause marked effects throughout an ecosystem.

Mycorrhizal Production. Mycorrhizal symbiosis is an important process in most ecosystems where rooted plants exist. Mycorrhizal associations and nutrient availability are strongly related. Nutrient or water limitation increases mycorrhizal development, in turn increasing the plant's competitive ability for minerals. Addition of nutrients can greatly reduce mycorrhizal associations (Bowen 1981,1984). Reducing mycorrhizae by adding nitrogen would reduce plant ability to obtain other resources (e.g. phosphorus or water). Mycorrhizae figure importantly in plant-plant competition for nutrients and water and evidence suggests they are also involved in allelopathic interactions between plants (St. John and Coleman 1983). Others postulate that mycorrhizal fungi influence the decomposition of plant detritus by suppression of other microbes active in decomposition (Gadgil and Gadgil 1975). In the long term, this may be

important in the formation and accumulation of humus layers.

Stress Tolerance. Biotic and abiotic stress factors occur to varying degrees in all ecosystems. Commonly reported are stresses due to drought, acidity, salinity, temperature, and heavy metals. Species composing particular ecosystems have experienced the selection pressures resulting from such stresses and have adapted. Manipulating stress factors or an organism's tolerance-adaptation to a stress should change competitive relations within the community. There is a great deal of interest in GEOs that are more tolerant of these stresses, or more productive in spite of the stresses. Eventually, GEOs may directly affect species interactions in natural ecosystems.

Growth Rate. Competitive interactions among species are related to growth rates, especially in the plant community. Altered growth rates resulting from any of the above-discussed factors or from direct manipulation of growth substances (e.g. auxins, gibberillin, florigen, etc.) would be expected to influence competitive interactions.

B. Oxidation (Catabolic) Processes:

Organic Matter Decomposition. One dominant characteristic of any ecosystem is the amount of detrital organic matter which has accumulated on and in soils and sediments and has dissolved in water. This material greatly affects the physical, chemical, and biological properties of the ecosystem. Decomposition and organic matter accumulation are most sensitive to environmental and biological variation. The increased decomposition of organic matter in agricultural soils is a striking example. Decomposition rates could be influenced by some of the proposed GEOs (e.g. toxic waste degraders). Some obvious consequences of enhanced rates of decomposition of this detrital material would include changes in microbial and invertebrate biomass and populations, nutrient availability and cycling rates, soil physical structure, quality of the organic matter, oxygen availability, water holding capacity,

mineral weathering rates, heavy metal levels, acidity, soil temperature and its variation, and competitive interactions among species, to name a few.

Nitrification. Nitrification is a key process, acting either positively or negatively, depending on the ecosystem under consideration. On the negative side, nitrification results in lowered pH and decreased oxygen. High nitrate concentrations can reduce microbial activity and carbon turnover in soil, degrade groundwater quality, promote cations leaching from soil, and aggravate certain plant diseases. Nitrification in aquatic ecosystems, by producing a mobile, water-soluble form of nitrogen, can contribute to eutrophication, and by producing nitrous oxide, a key gas in the changing tropospheric chemistry and possible global warming, may have far reaching effects indeed. On the positive side, heterotrophic nitrifiers produce certain growth factors and antibiotics and autotrophic nitrifiers may be instrumental in reducing ammonium accumulations (ammonium toxicity) in some environments. In addition, nitrification supplies nitrogen to plants in the anionic form which can enhance the uptake of other essential cations. The growth of young plants, in particular, is promoted by the nitrate form, although some plants prefer nitrate to ammonium throughout their life cycle. In general, nitrification has generally been considered a positive process relative to soil fertility (Verstraete 1981). The conflicting positive and negative attributes of nitrification have clouded the issue of whether nitrification should be protected from such things as pesticides or controlled or even totally inhibited through agricultural practices and chemicals.

In natural ecosystems, the rate of nitrification often is depressed. Some scientists believe that minimal rates of nitrification are important for nutrient preservation. The mechanisms controlling nitrification in natural ecosystems are not exactly known; however, ammonium limitation has received most support. Nitrifiers are believed to be relatively poor competitors for

ammonium with plants and other microbes and under nitrogen limitation, the process is minor. Substantial support also exists for organic chemical inhibition of nitrifiers in some ecosystems or under certain conditions (Verstraete 1981). Regardless of its positive or negative effect, the rate of the process may be changed if nitrogen is significantly increased in a system (e.g. N fixation), or if organic inhibitors are produced or degraded. Genetic engineering has the potential to alter nitrification rates through these and other means.

C. Disease and Pest Control:

Species Interactions. This topic has been discussed relative to other anabolic and catabolic processes. Other genetic manipulations are being researched which interfere directly with species interrelationships. Much interest focuses on disease and pest control because of the problems they cause for human society. Many natural insecticides, fungicides, and bactericides occur, the result of eons of evolution. Sudden changes in their levels, placement in new situations or new species, etc., would naturally alter relations of organisms in ecosystems, including non-target organisms. The genes for the production of naturally-occurring microbial insecticides are likely to be some of the first released into the environment in GEMs.

PERTURBATION BY GENETICALLY ENGINEERED ORGANISMS

A. Production (Anabolic) Processes:

Nitrogen Limitation. Improving the nitrogen status primarily involves increasing nitrogen-fixation which may be done in a number of ways, exemplified by the following list:

1) Broadcast application of free-living genetically engineered indigenous microorganisms.

   a) Pseudomonad with nitrogen-fixing gene complex (NIF)

   b) Free-living Pseudomonad with added NIF plus cellulase gene

c) Autotrophic microorganism with added NIF

d) Native autotrophic N-fixer with genes for extending the tolerance range for a particular stress (e.g. temperature, acidity)

2) Add NIF gene complex directly to crop plant.

This list represents a range of probabilities for nitrogen addition to the system and accidental escape of the genetic material to other organisms/ecosystems. The possibility of accidental lateral transfer of genetic material is greatest for microorganisms and least for a vascular plant. However, the probability of successfully increasing the nitrogen levels in the system is lowest for 1) above and greatest for 2) because nitrogen fixation is an energy demanding process (10 to 20 molecules of ATP for every molecule of nitrogen fixed or about 2.6 grams of carbon per gram of nitrogen fixed). Free-living Pseudomonads would be subject to immediate secondary limitations of energy as well as phosphorus, molybdenum, iron, and stress. Catagories 1 (b,c,d) represent more complex genetic engineering in that the microorganism also has its own energy source (e.g. cellulase activity, autotrophy) and is more tolerant of secondary limitations. Thus, the list from a to d represents increasing probability of success in fixing nitrogen in the system. The list represents a decreasing probability of lateral transfer of the genetic material to make successful non-target species. Direct placement of the NIF gene complex in a crop plant, though technically most difficult, represents the greatest probability of increasing nitrogen availability and lowest probability of accidental 'escape' of the genetic material. For example, if the NIF gene complex was placed in corn, the most probable 'escape' potential would be through pollen dispersal. The unrelated nature of most native species would prevent fertilization by corn pollen and transfer of its genetic material.

The probability of success for nonautotrophic microorganisms is directly related to the available carbon in the habitat. If the GEM has cellulase

activity, habitat areas such as littoral zones, estuarine areas, eutrophic waters, organic-rich surface soils, rhizosphere, etc. would be candidates for successful establishment of the new organism.

The literature discussed under nitrogen cycling identified potential ecological impacts if nitrogen levels are increased in an ecosystem. Below we rank those effects most likely to ensue (1) and then others in descending order of likelihood.

1) a) Increased leaf and shoot production in target plants
   b) Decreased root growth and biomass
   c) Increased decomposition rates in some systems
   d) Increased ammonification and nitrification in soil
   e) Increased competition on non-nitrogen fixing plants and alteration of plant community structure with ensuing reverberations throughout the indigenous biota.

2) a) Decreased decomposition rates in some systems (Flanagan and Van Cleve 1983)
   b) Increased potential for other element limitations especially if a) results
   c) Altered Cation exchange capacities and pH shifts.

3) a) Decreased mycorrhizal associations (dependent on 2b) above)
   b) Shift from carbon-based plant defense to nitrogen based defense (or from immobile to mobile defense compounds).
   c) Altered foliage tissue palatability and herbivory (dependent on 3b)).
   d) Increased terrestrial denitrification rates and increased emissions of N gasses. (dependent on 1d) and 2c) above).

4) These effects would be more or less likely dependent on a site's proximity to water. Obviously, in desert or xeric systems they would be

minimal.

a) Increased leaching loss of nitrate.

b) Increased eutrophication of drainage water.

c) Increased algal production in receiving waters.

d) Increased carbon enrichment of aquatic sediments.

e) Increased anaerobic conditions in aquatic habitats.

f) Increased aquatic nitrification and denitrification rates and gaseous emissions to the atmosphere.

These represent directional shifts in processes and may or may not all occur in given situations.

Phosphorus Limitation. The primary means of increasing phosphorus availability will involve stimulating phosphatase activity in such ways as:

1. Widespread applications of free-living genetically engineered, indigenous microorganisms.
   a. Broadcasting mycorrhizal fungi or bacteria with enhanced phosphatase activity.
   b. Stimulating gene regulation sequences for phosphatase activity in native microflora.
2. Stimulation of phosphatase activity directly in plants.

The probability of lateral transfer of genetic material in these cases parallels those of nitrogen fixation. Direct insertion or stimulation of phosphatase activity in plants has the highest probability for success in increasing phosphorus availability and the lowest probability for accidental release and horizontal transfer in the environment. The case is reversed in broadcasting free-living microbes with enhanced phosphatase activity. Likely applications of this technology could be in association with plants receiving the NIF gene complex, agricultural applications where high-phosphorus requiring products such as seeds, flowers, bulbs, fruit, etc., are desired, and in

sedimentary rock-derived and marginal-fertility soils where phosphorus supplies are low. Enhanced phosphatase activity in sites may best be achieved by genetic manipulation of plants and/or through mycorrhizal associations with gene-altered mycosymbionts.

Increases in phosphorus availability and cycling may lead to the following responses within terrestrial and aquatic ecosystems:

As with N, the effects below are ranked in decreasing order of likelihood First, we list possible effects of increasing phosphatase (phosphorus availability) alone, and then of increasing phosphatase and N fixation in one system simultaneously.

Increasing phosphorus availability (alone)

1)Most likely

a) Increased target plant leaf and shoot growth / decreased root growth.

b) Decreased competition with non-phosphatase enhanced plants with ensuing plant community structural changes and changes in indigenous biological community.

c) Increased decomposition rates

d) Decreased mycorrhizal associations

e) Increased target plant reproductive capacity exacerbating 1b) above.

2) a) Decreased decomposition rates (Flanagan and Van Cleve, 1983) exacerbating 1b) **or**

b) Increased potential for other element limitation (e.g. Ca, N, K, and trace elements).

3) As with nitrogen, these effects diminish in likelihood with distance from water bodies.

a) Accelerated eutrophication of ground waters and runoff

leading to the same in lakes, rivers and reservoirs.

b) Increased algal production particularly favoring N-fixing algae.

c) Increased hydrophyte production, anaerobic conditions, and gaseous emissions in littoral zones of aquatic systems.

Comparison of putative consequences with enhancement of N and P separately, and by combining effects for both which could ensue from genetic insertion of factors enhancing, at once, N fixation and phosphatase activity, leads to the following potential effects; again in decreasing order of likelihood for terrestrial ecosystems (but now the effects are at least twice as likely to occur).

1) Most likely

a) Increased target plant leaf and shoot production.

b) Increased target plant reproductive capacity.

c) Increased competitive ability of target plants with ensuing plant community structural shifts and ecological reverberations throughout populations communities, and trophic orders.

d) Decreased root growth and biomass in target plants.

e) Decreased target plant symbiotic root associations (mycorrhizae and bacteria).

f) Increased ammonification and nitrification.

2) a) Shifts in quantity and quality of plant defense chemicals.

b) Changes in herbivory participants and degree.

c) Altered decomposition rates and/or organisms.

d) Increased denitrification (N increase and N_2O increase to atmosphere)

3) Those effects listed for aquatic systems above would be twice as

likely to occur, and with at least twice the intensity ensuing from increasing just N or P alone.

The above lists represent potential shifts in the plants, microbes, and processes in various ecosystems which could be manifested to varying degrees. Many of the effects may be advantageous for agricultural production but deleterious for some natural terrestrial ecosystems, in the sense of causing change. The proposed effects may be considerably more deleterious to aquatic ecosystems. Research which considers both the terrestrial and aquatic ecosystem response would be appropriate to address the effects of genetic engineering focused on genes for nitrogen fixation and/or phosphatase activity.

Mycorrhizal Production. Mycorrhizae benefit the host plant principally by increasing the root absorbing area for nutrients and water. As pointed out already, high nutrient availability can decrease mycorrhizal formation and plant root mass. Optimum crop production may require extensive mycorrhizal association, and genetic engineering may be the procedure to procure it.

The response of a plant to inoculation with mycorrhizal fungi depends on fertility of the site, population levels of naturally occurring mycorrhizae and the effectiveness of the mycorrhizal fungi relative to other fungi. There may be a several-fold difference in the absorption capability for a nutrient such as phosphorus between mycorrhizae formed by different fungi. Different fungi stimulate plant growth to different extents, thus, plant growth can be enhanced by inoculation with selected fungi. Some plant species are very specific with regard to fungal species that can develop mycorrhizae while others are not. Genetic engineering may be used to control (increase) infection rates with specific fungi. Genetic engineering also may allow the addition of nitrogen-fixing capabilities similar to associations that form naturally with some beans and other legumes which are notable in forming symbioses with mycorrhizal fungi and Rhizobium. These associations have improved growth

rates due to both their nitrogen-fixing ability and high root absorbing capacity for phosphorus and micronutrients. Such associations should be successful in improving plant growth rates and have minimal risk in altering the genetic material of non-target organisms. The results may be an overall increase in nutrient availability in the system and trends similar to those discussed for nitrogen and phosphorus.

Stress Tolerance and Growth Rate. We envision these manipulations to be performed on specific crop species although a microorganism that can perform a specific function may be engineered to increase it's habitat range. In crop species, the risk of lateral transfer to other organisms is relatively low. If the crop species is a native species (e.g. timber-producing tree species), competitive displacement may be the most significant response. Enhanced growth rates may result in higher uptake of nutrient resources and the harvest of the crop may result in nutrient depletion at an accelerated rate. This would lead to predictions that were opposite those listed for increased nitrogen and phosphorus availability and cycling.

B. Oxidation (Catabolic) Processes:

The degradation of xenobiotics, toxic wastes, and various organic and inorganic pollutants represents an area of high visibility and activity for genetic engineering. Microorganisms will be the likely vehicle; however, their use increases the probability of unplanned spread to native species and ecosystems (Freter 1984). The procedure for degradation of these compounds also will involve a broadcast strategy and use of large numbers of GEMs which increases the potential for interactions with indigenous microbes.

Native microorganisms can degrade a wide range of organic compounds, but their rates of degradation and breadth of usable compounds can be expanded through culture techniques and genetic manipulation. For example, lignin degradation is a slow process because initial breakdown by a specific enzyme

results in repolymerization of residual or waste products that the organism cannot break down further because of a lack of appropriate enzymes. A succession of other species with appropriate enzymes is required to break down the sequence of polymers produced. It is likely that an organism will be engineered with a suite of enzymes (i.e. complex of polyphenol oxidases), allowing a relatively complete and rapid breakdown by a single organism (super bug concept). The escape and establishment of such organisms in natural ecosystems may result in profound changes. The transfer of a complex of genes is less probable than single gene transfers; however, there are numerous reports of E. coli and coliform bacteria carrying transferable multiple antibiotic resistance markers in sewage and open waters (Freter 1984). The following discussion assumes that GEMs will escape and be successful in other environments, and/or that novel genes, gene complexes, and gene regulatory sequences will be transferred. The discussion concentrates on likely effects.

The recent literature is rich in reports of native microbial isolates capable of degrading a wide variety of xenobiotic compounds and/or industrial chemicals (e.g. Ghosal et al. 1985). These include such diverse products as herbicides and pesticides (e.g. McBride et al. 1986), industrial solvents (e.g. Strand and Shippert 1986, Schraa et al. 1986), wood preservatives (e.g. Brown et al. 1986, Mikesell and Boyd 1986), plasticizers (e.g. Dwyer and Tiedje 1986), dyes (e.g. Nortemann et al., 1986), etc. Isolates usually have been cultured from environments where the compounds occur in high concentration and further manipulations in the lab have enhanced the degradative capability of the culture. A wide variety of organisms including aerobic and anaerobic heterotrophs, methanogens, denitrifiers, and sulfate reducers have been used to decompose various organic pollutants. Reauthorization on October 17, 1986, of the Superfund program for 9 billion dollars to begin cleanup of the 375 worst toxic waste sites by 1991 guarantees that intensive research on microbial

decomposers of various forms of the toxic waste will continue.

Plasmid-assisted molecular breeding has already produced bacterial strains capable of totally degrading herbicide 2,4,5-T, the active compound in Agent Orange (Kellogg et al. 1981). The diversity of xenobiotic pollutants which can be degraded by known bacterial strains inevitably will lead to further research on genetic recombinant procedures to engineer organisms capable of degrading multiple contaminants in soil and water. The probability of such organisms being produced is high and their use will require widespread environmental release of these strains. Direct introduction of these organisms into soil, groundwater, and surface waters is likely in the near future.

An organism with engineered capability to degrade an array of xenobiotic compounds will require complexes of enzymes such as polyphenol oxidases to initiate the decomposition of recalcitrant aromatic structures common to many xenobiotics. This ability to mineralize such chemical structures may enhance the decomposition of humic and fulvic acids in terrestrial and aquatic ecosystems. A number of structural similarities exist between naturally occurring humic materials and many of the aromatic xenobiotics present at toxic waste sites (e.g. Christman and Gjessing 1983, Aiken et al. 1985, and Thurman 1985). An increase in the rate of humic material breakdown is a possibility from introduction of GEMs capable of breaking down an array of chemical pollutants.

If the rate of breakdown of soil humic material increases, a number of physical and chemical changes would occur. Physical changes would include decreased cation exchange capacity, decreased water holding capacity, and increased soil erosion. Chemical changes would include a decrease in the litter layer, a decrease in the total soil organic matter and an increase in carbon dioxide, available N and P, and soluble organic carbon. In the short term, these changes would be predicted to yield an increase in productivity.

Long-term response could be a net decrease in productivity as the storehouse of energy and nutrients in the soil dwindles.

An increased rate of decomposition of detrital organic matter in aquatic ecosystems similarly would boost productivity as greater quantities of organic carbon, N and P are made available for uptake and utilization. Accelerated rates of eutrophication could result. Wetzel (1983) argues that the large supply of recalcitrant detrital material in freshwater ecosystems lends stability to the ecosystems. Changing the decomposition rate of this material would affect net ecosystem productivity and the availability of oxygen in zones where reaeration is slow. Zones of anaerobic conditions within water-logged soils, sediments, and bottom waters in stratified lakes would expand. Anaerobic pathways of decomposition such as denitrification, sulfate reduction, and methanogenesis would replace aerobic pathways in these zones and both the rate and overall extent of these processes would increase. The rate of production of methane in freshwater ecosystems low in nitrate and sulfate would increase. Greater carbon dioxide and methane input to the atmosphere is a predicted end result of the successful permanent establishment of a GEM with better capability to decompose recalcitrant xenobiotics and naturally-occurring humic materials.

A ranked summary of the potential responses within terrestrial and aquatic ecosystems to increased rates of microbial decomposition of humic material includes:

1) a) Decreased litter accumulation
   b) Decreased total organic matter of soils and sediments
   c) Increased C, N, and P availability (short term)
   d) Decreased cation exchange capacity
   e) Decreased water holding capacity
2) a) Increased plant productivity (to begin with but won't last)

b) Resetting of successional phases in natural systems

c) Increased CO_2 and methane flux

3) a) Increased soil erosion

b) Increased anaerobic decomposition and anaerobic conditions

c) Decreased complexation of metals and proteins in organic matter.

The likelihood of such changes in ecosystem characteristics and processes actually occurring depends on the GEMs either directly increasing the rate of detrital decomposition or transferring this capability to native microflora. The rate of decomposition of naturally occurring humic substances by GEMs and the probability of transfer of the engineered degradative capability to indigenous microbial populations are key areas where research will be required.

C. Disease and Pest Control:

Control of diseases and pests via genetic engineering also is receiving high visibility and a number of examples are available. A Bacillus thuringiensis gene has been transferred into Psuedomonas syringae that produces an endotoxin against rhizosphere lepidoptera larvae. Some British work involves the use of a virus for control of a parasitic moth larvae on pine. The U.S. has genetically engineered viruses for control of cattle diseases, which already have been released in Argentina and New Zealand. Where these genes can be engineered directly into the plant for pest control, the escape risk is reduced. However, microorganisms used as the toxin producing agent have the potential for lateral transfer as described in other sections. As evidenced by the buildup of multiple antibiotic resistant strains of microorganisms, there may be strong selection pressure for resistant strains to any genetically-engineered endotoxin. There would have to be a continual development of new endotoxins much in the same way there is a continual development of crop strains resistant to rapidly evolving pests and diseases.

Other concerns involve the accumulation of toxins in the environment.

Toxins produced by organisms are more likely to be degraded than novel, chemically-derived compounds. For example, the Bacillus thuringiensis endotoxin is labile in ultraviolet light and has a very short half life. There are, however, a number of mechanisms that could result in toxin accumulation. Abundant literature exists on the accumulation of enzymes sequestered in mineral-organic complexes in the soil (i.e. soil enzymes). These extracellular enzymes are very important to the functioning of microorganisms and plants in an ecosystem. The protein-based endotoxins may have the same capability for accumulation. It is difficult to predict the result of an accumulation. It could result in a general depression of microbiological activity or very species-specific effects depending on the toxin involved. Some species may be the agents of accumulation by a buildup of the toxin in their body tissues. The most significant effect may be altered predation rates on those organisms with a buildup of toxin and a change in the competitive relations among organisms.

RECOMMENDATIONS

This section will summarize our recommendations to increase predictive capability of risk assessment and identify priority areas for research.

Facilities Development:

Our literature review identified the potential for a large range of results across many disciplines that could occur from the release of GEOs. Thus, we make a strong plea for an interdisciplinary research program. We need more than just a number of different disciplines working on a research topic. The research must be thoroughly integrated from the level of hypothesis generation to experimentation and interpretation of results. This can be done best at a research "center" where logistical constraints are minimized. We envision a "center" as a consortia of government, industry, and academia located at a university. The "center" should be government and industry

funded.

The research needed will require dedicated major equipment and facilities (e.g. laboratories, enclosed greenhouses) which can be used by interdisciplinary research teams. This will be possible at relatively few locations. The location of the "center" should be given serious attention. It may be desirable to locate at least one "center" in a "hostile" environment (e.g. arctic tundra, hot desert) to minimize the chance of accidental escape. These "centers" may concentrate on GEOs that present a higher risk potential.

The "centers" could provide a variety of functions in addition to facilitating basic research. They could develop techniques and instrumentation, produce GEOs for specific purposes, screen GEOs for environmental effects, and be an information source for regulatory agencies.

General Research Objectives:

In addition to our strong plea for interdisciplinary research, we feel the research must go beyond the microcosm level. We must approach 'real world' scales of complexity that exist at the community and ecosystem levels. Experimental design must accomodate these different scales of research. For example, a research problem should couple both microcosm and mesocosm (i.e. enclosed greenhouses with realistic communities of organisms) approaches. If a research stage involves a field release of a GEO, the site of release must be well studied and its natural history well established with an inplace monitoring system that has been well-tested before the release. The research must go beyond that of organism interactions. Results should include process-level and ecosystem-level interactions to help evaluate larger scale and longer term effects.

Most planned introductions of GEOs will take place in agricultural settings. Application in one ecosystem does not preclude movement of the organism or capability into other ecosystems. In particular, beneficial

attributes in food production such as enhanced growth, introduced nitrogen-fixing capability, increase phosphatase activity, and better pest management may be undesirable in certain aquatic ecosystems. Terrestrial ecosystems and the streams, ponds, lakes, and reservoirs that drain them are closely coupled. The interdisciplinary research which is required must not set up artificial ecosystem boundaries, but instead, remain cognizant that ecosystems are strongly interlinked. Therefore it is important that an appreciation of landscape ecology exist.

A major question in this research will involve developing proof that a significant change is a GEO-mediated change. The answer requires integrated research at population, community and process levels. Research by Schindler et al. (1985) in an artificially acidified lake over almost a decade showed that process-level phenomena did not change markedly even though some species population changes were dramatic. Many higher tropic species were lost from the lake and some new species were added, but the dominant processes changed only slightly. Over a longer time period, process level phenomena may change markedly and in a way that is not correlated with short term population changes. The process level changes may be a result of the sequence or accumulation of short-term population changes (Strayer et al. 1986). Process level changes may be influenced largely by altered microbial dynamics that are not strongly correlated with high trophic level (predator) populations.

Risk Assessment:

We believe it is important to develop a classification based on risk as soon as possible. This risk classification will be important in identifying the controls necessary for evaluating suspected consequences of GEO releases. For example, plasmid transfer among microorganisms is well established and constitutes a high risk for transfer to native microorganisms after release. The initial research for this type of work must be strongly-controlled

microcosm work. Genetically engineered microorganism research, in general, should be carefully considered and initiated with microcosm work. In contrast, new techniques such as electroporation may be able to put gene(s) directly into plants and animals and minimize the risk of escape. Genetic exchange potentials between plants and animals is much lower than for microorganisms; however, retrovirus research indicates that horizontal exchange can occur. Some engineered plants represent relatively low risk and research may be able to start at the mesocosm or field release stage (e.g. NIF gene complex in corn). Engineered plants that have the same or closely-related species in nearby natural environments should be researched in mesocosms. All research does not have to start at the microcosm level.

Specific Research Topics:

The areas needing research in risk classification of GEO releases are virtually limitless. However, there are several specific areas that need immediate attention to help begin plan the overall research program. Ranked in our opinion of their priority are:

1. Develop the capability for mesocosm research where a realistic assemblage of organisms, both terrestrial and aquatic, can be maintained under controlled but natural conditions and used for research on GEOs soon to be released into the environment.
2. Measure breakdown rates of native detritus in soils, sediments, and waters by genetically-engineered xenobiotic degraders.
3. Measure the magnitude of increase in nitrogen and phosphorus availability that is attainable by genetic engineering under semi-natural conditions (micro- and mesocosm conditions).
4. Measure the affect on nitrification rates from changing nitrogen and/or phosphorous levels or from altering nitrifying organisms.
5. Demonstrate changes in nutrient loss rates in micro- and mesocosms by

measuring any of above changes.

6. Demonstrate competitive displacement among native organisms in mesocosms by manipulating: a)resource availability; b) endotoxins; c) stress tolerance.

7. Demonstrate a relationship between population-level changes and ecosystem-process changes in mesocosms.

8. Demonstrate system-level increases in trace gas flux to the atmosphere (e.g. CO_2, CH_4, N_2O) in micro- and mesocosms.

It is no accident that this list contains so much research on microbes. Microbial ecology is an emergent facet of ecology. The dearth of data, understanding and functional models in this sub-discipline urgently needs to be addressed nationally. Risks from GEOs will continue and increase until we know how regenerative soil, sediment, and water processes work and how these procesees are controlled or regulated by microbial communities.

REFERENCES

Agren, G.I., Axelsson, B., Flower-Ellis, J.G.K., Linder, S., Persson, H., Staaf, H. & Trueng, E. (1980). Ecol. Bull. (Stockholm), 32: 307-313.

Aiken, G.R., McKnight, D.M., Wershaw, R.L. & MacCarthy, P. (1985). "Humic Substances in Soil, Sediment, and Water". Wiley-Interscience.

Aumen, N.G., Bottomley, P.J., Ward, G.M. & Gregory, S.V. (1983). Appl. Environ. Microbiol. 46: 1409-1416.

Bell, F.C. (1979). In "Arid Land Ecosystems: Structure, Functioning and Management", (D.W. Goodall & R.A. Perry, eds.), pp. 373-392. vol. 1. Cambridge University Press, Cambridge.

Benoit, R.E. & Starkey, R.L. (1968a). Soil Sci. 105: 203-208.

Benoit, R.E. & Starkey, R.L. (1968b). Soil Sci. 105: 291-296.

Benoit, R.E., Starkey, R.L. & Basaraba, J. (1968). Soil Sci. 105: 153-158.

Billen, G. (1982). Am. J. Sci. 282: 512-541.

Bolin, B. (1970). Sci. Am. 223: 125-132.

Bowen, G.D. (1981). In "Terrestrial Nitrogen Cycles", (F.E. Clark & T. Rosswall, eds.), pp. 237-248. Ecol. Bull. (Stockholm), No.33. SCOPE.

Bowen, G.D. (1984). In "Nutrition of Plantation Forests", (G.D. Bowen & E.K.S. Nambiar, eds.), pp. 147-180. Academic Press, New York.

Broeker, W.S. & Peng, T.H. (1982). "Tracers in the Sea". Eldigio.

Brown, E.J., Pignatello, J.J., Martinson, M.M. & Crawford, R.L. (1986). Appl. Environ. Microbiol. 52: 92-97.

Button, D.K. (1984). Appl. Environ. Microbiol. 48: 1004-1011.

Capone, D.G. & Carpenter, E.J. (1982). Science 217: 1140-1142.

Carey, M.L., Hunter, I.R., Thorn, A.J. & Andrew, I.A. (1982). N.Z. J. For. Sci. 12: 36-48.

Chatarpaul, L., Robinson, J.B. & Kaushik, N.K. (1980). Can. J. Fish. Aquat. Sci. 37: 656-663.

Chen, K.Y. & Morris, J.C. (1972). Environ. Sci. Technol. 6: 529-537.

Christman, R.F. & Gjessing, E.T. (1983). "Aquatic and Terrestrial Humic Materials". Ann Arbor Science.

Coley, P.D., Bryant, J.P. & Chapin, F.S., III. (1986). Science 230: 895-899.

Crawford, C.S. & Gosz, J.R. (1982). Environmental Conservation. 9: 181-195.

Cummins, K.W. (1974). Bioscience 24: 631-641.

Dahm, C.N., Baross, J.A., Ward, A.K., Lilley, M.D. & Sedell, J.R. (1983). Appl. Environ. Microbiol. 45: 1633-1645.

Davies, R.I., Coulson, C.B. & Lewis, D.A. (1964). J. Soil Sci. 15: 299-309.

Delwiche, C.C. (1977). Ambio. 6: 106-111.

Dwyer, D.F. & Tiedje, J.M. (1986). Appl. Environ. Microbiol. 52: 852-856.

Edmondson, W.T. (1970). Science 169: 690-691.

Edmondson, W.T. & Lehman, J.T. (1981). Limnol. Oceanogr. 26: 1-29.

Fisher, S.G. & Likens, G.E. (1973). Ecol. Monogr. 43: 421-439.

Flanagan, P. (1986). Bull. Ecol. Soc. Amer. 67: 26-30.

Flanagan, P.W. & Van Cleve, K. (1983). Can. J. For. Res. 13: 795-817.

Florence, R.G. & Lamb, D. (1974). N.Z. J. For. Sci. 11: 425-433.

Freter, R. (1984). In "Current Perspectives in Microbial Ecology", (M.J. Klug & C.A. Reddy, eds.), pp. 105-114. Amer. Soc. Microbiol., Washington, D.C.

Gadgil, R.L. & Gadgil, P.D. (1977). N.Z. J. For. Sci. 5: 33-41.

Gardner, W.S. & Scavia, D. (1981). Limnol. Oceanogr. 26: 801-810.

Ghosal, D., You, I.S., Chatterjee, D.K. & Chakrabarty, A.M. (1985). Science 228: 135-142.

Goldman, C.R. (1981). Verh. Int. Ver. Limnol. 21: 45-70.

Goldman, C.R. & Horne, A.J. (1983). "Limnology". McGraw-Hill.

Gosz, J.R. (1978). Water Research 12: 725-734.

Gosz, J.R. (1981). In "Terrestrial Nitrogen Cycles", (F E. Clark & T. Rosswall, eds.), pp. 405-426. Ecol. Bull. (Stockholm) No. 33. SCOPE.

Gregory, S.V. (1980). Ph.D. Dissertation, Oregon State Univ. Corvallis, Ore.

Grier, C.C., Vogt, K.A., Keyes, M.R. & Edmonds, R.L. (1981). Can. J. For. Res. 11: 155-167.

Grimm, N.B. & Fisher, S.G. (1986). J. N. Amer. Benthol. Soc. 5: 2-15.

Haines, E.B. (1977). Oikos 29: 254-260.

Harris, W.F., Kinerson, R.S. & Edwards, N.T. (1977). In "The Below Ground Ecosystem: A Synthesis of Plant-Associated Processes", (J.K. Marshall, ed.), pp. 29-37. Colorado State Univ., Ft. Collins, CO.

Hermann, R.K. (1977). In "The Below Ground Ecosystem: A Synthesis of Plant-Associated Processes", (J.K. Marshall, ed.), pp. 7-28. Colorado State Univ., Ft. Collins, CO.

Herrera, R. & Jordan, C.F. (1981). In "Terrestrial Nitrogen Cycles", (F.E. Clark, & T. Rosswall, eds.), pp. 493-505. Ecol. Bull. (Stockholm) No. 33. SCOPE.

Hill, A.R. (1983). In: "Dynamics of Lotic Systems", (W.T. Fontaine III & S.M. Bartell, eds.), pp. 457-477. Ann Arbor Science.

Horne, A.J. & Carmiggelt, C.J.W. (1975). Freshwater Biol. 5: 461-470.

Howarth, R.W. & Hobbie, J.E. (1982). In "Estuarine Comparisons", (V.S. Kennedy, ed.), Academic Press.

Howes, B.L., Dacey, J.W.H., & King, G.M. (1984). Limnol. Oceanogr. 29: 1037-1051.

Howes, B.L., Dacey, J.W.H. & Teal, J.M. (1985). Ecology 66: 595-605.

Jackson, G.A. (1980). Nature 284: 439-441.

Jenkins, M.C. & Kemp, W.M. (1984). Limnol. Oceanogr. 29: 609-619.

Jones, M.B. & Woodmansee, R.G. (1979). Bot. Rev. 45: 111-144.

Kellogg, S.T., Chatterjee, D.K. & Chakrabarty, A.M. (1981). Science 214: 1133-1135.

Koike, I., Nishio, T. & Hattori, A. (1983). In "Current Perspectives in Microbial Ecology", (M.J. Klug & C.A. Reddy, eds.), Amer. Soc. Microbiol., Washington, D.C.

Larcher, W. (1975). "Physiological Plant Ecology". Springer-Verlag, New York.

Lerman, A., MacKenzie, F.T. & Garrels, R.M. (1975). Geol. Soc. Am. Mem. 142: 205-218.

Likens, G.E. (1981). "Some Perspectives on the Major Biogeochemical Cycles". John Wiley & Sons.

Likens, G.E., Bormann, F.H., Johnson, N.M., Fisher, D.W. & Pierce, R.S. (1970). Ecol Monogr. 40: 23-47.

Likens, G.E., Bormann, F.H., Pierce, R.S. & Eaton, J.S. (1977). "Biogeochemistry of a Forested Ecosystem". Springer-Verlag, New York.

MacGregor, A.N. & Johnson, D.E. (1971). Soil Sci. Soc. Proc. 35: 843-844.

Martinez, L.A., Silver, M.W., King, J.M. & Alldredge, A.L. (1983). Science 221: 152-154.

Maybeck, M. (1982). Am. J. Sci. 282: 401-450.

McBride, K.E., Kenney, J.W. & Stalker, D.M. (1986). Appl. Environ. Microbiol. 52: 325-330.

Meentemeyer, V. (1978). Ecology 59: 465-472.

Melillo, J.M. & Gosz, J.R. (1983). In "The Major Biogeochemical Cycles and Their Interactions", (B. Bolin & R.B. Cook, eds.), pp.177-222. John Wiley & Sons, New York.

Meyer, J.L., Likens, G.E. & Sloane, J. (1981). Archiv. fur Hydrobiol. 91: 28-44.

Mikesell, M.D. & Boyd, S.A. (1986). Appl. Environ. Microbiol. 52: 861-865.

Naiman, R.J. & Sibert, J.R. (1979). J. Fish. Res. Board Can. 36:504-520.
Newbold, J.D., Elwood, J.W., O'Neill, R.V. & VanWinkle, W. (1981). Can. J. Fish. Aquat. Sci. 38: 860-863.

Nishio, T., Koike, I. & Hattori, A. (1983). Appl. Environ. Microbiol. 45: 444-450.

Nortemann, B., Baumgarten, J., Rast, H.G. & Knackmuss, H.J. (1986). Appl. Environ. Microbiol. 52: 1195-1202.

Noy-Meir, I. (1973). Ann. Rev. Ecol. Syst. 4: 25-51.

Noy-Meir, I. (1974). Ann. Rev. Ecol. Syst. 5: 195-213.

O'Brien, T.F., Pla, M.D.P., Mayer, K.H., Kishi, H., Gilleece, M. Syvanen, M. & Hopkins, J.D. (1985). Science 230: 87-88.

Parsons, T.R. & Takahashi, M. (1973). "Biological Oceanographic Processes". Pergamon Press.

Paul, E.A. (1976). Environ. Biochem. 1: 225-243.

Probyn, T.A. & Painting, S.J. (1986). Limnol. Oceanogr. 30: 1327-1331.

Redfield, A.C. (1958). Am. Sci. 46: 205-222.

Riley, G.A. (1963). Limnol. Oceanogr. 8: 372-381.

Rodin, L.E. & Bazilevich, N.I. (1967). "Production and Mineral Cycling in Terrestrial Vegetation". Edinburgh and London: Oliver and Boyd.

Rosenzweig, M.L. (1968). Amer. Nat. 102: 67-74.

Rosswall, T. & Granhall, U. (1980). In "Ecology of a Subarctic Mire", (M. Sonesson, ed.), pp. 209-234. Ecol. Bull. (Stockholm) vol. 30.

Rowan, S.J. (1970). Phytopathology 60: 1221-1224.

Rychert, R.C. & Skujins, J. (1974). Soil Sci. Soc. Am. Proc. 38: 768-771.

Rychert, R.C., Skujins, J. & Porcella, D. (1978). In "Nitrogen in Desert Ecosystems", (N.E. West & J.J. Skujins, eds.), pp. 20-30. (US/IBP Synthesis Ser. 9), Dowden, Hutchinson & Ross, Stroudsburg, Penn.

Ryther, J.H. (1969). Science 166: 72-76.

Ryther, J.H. & Dunstan, W.M. (1971). Science 171: 1008-1013.

Scarsbrook, C.E. (1965). In "Soil Nitrogen", (W.V. Bartholomew & F.E. Clark, eds.), pp. 481-502. Agronomy No. 10. Madison, Wisc.

Schindler, D.W. (1971). J. Phycol. 7: 321-329.

Schindler, D.W. (1974). Science 184: 897-899.

Schindler, D.W. (1977). Science 195: 260-262.

Schindler, D.W., Mills, K.H., Malley, D.F., Findley, D.L., Shearer, J.A., Davies, I.J., Turner, M.A., Linsey, G.A. & Crnikshank, D.R. (1985). Science 228: 1395-1401.

Schraa, G., Boone, M.L., Jetten, M.S.M., van Neerven, A.R.W., Colberg, P.J. & Zehnider, A.J.B. (1986). Appl. Environ. Microbiol. 52: 1374-1381.

Simenstad, C.A. & Wissmar, R.C. (1985). Mar. Ecol. Prog. Ser. 22: 141-152.

Skujins, J. (1981). In "Terrestrial Nitrogen Cycles", (F.E. Clark & T. Rosswall, eds.), pp. 477-492. Ecol. Bull. (Stockholm). No. 33. SCOPE.

Slater, J.H. (1984). In "Current Perspectives in Microbial Ecology", (M.J. Klug & C.A. Reddy, eds.), pp. 87-93. Amer. Soc. Microbiol., Washington, D.C.

Smith, S.V. (1984). Limnol. & Oceanogr. 29: 1149-1160.

Soderlund, R. & Svensson, B.H. (1976). In "Nitrogen, Phosphorus and Sulphur - Global Cycles", (B.H. Svensson & R. Soderlund, eds.), pp. 23-73. SCOPE Report 7. Ecol. Bull. (Stockholm), vol. 22.

Sollins, P. & McCorison, F.M. (1981). Water Resources Research 17: 1409-1418.

Sollins, P., Cromack, K., McCorison, F.M., Waring, R.H. & Harr, R.D. (1981). J. Env. Qual. 10: 37-42.

Sorenson, J. (1983). In "Current Perspectives in Microbial Ecology", (M.J. Klug & C.A. Reddy, eds.), Amer. Soc. Microbiol., Washington, D.C.

St. John, T.V. & Coleman, D.C. (1983). Can. J. Bot. 61: 1005-1014.

Strand, S.E. & Shippert, L. (1986). Appl. Environ. Microbiol. 52: 203-205.

Strayer, D., Glitzenstein, J.S., Jones, C.G., Kolasa, J., Likens, G.E., McDonnell, M.J., Parker, G.G., & Pickett, S.T.A. (1986). "Long-term Ecological Studies: An Illustrated Account of Their Design, Operation, and Importance to Ecology". Occasional Publ. The Institute of Ecosystem Studies. No. 2.

Swift, M.J., Heal, O.W. & Anderson, J.M. (1979). "Decomposition in Terrestrial Ecosystems", Univ. of California Press, Berkeley, Calif.

Teal, J.M., Valiela, I. & Berlo, D. (1979). Limnol. Oceanogr. 24: 126-132.

Thomas, W.H. (1970a). Limnol. Oceanogr. 15: 380-385.

Thomas, W.H. (1970b). Limnol. Oceanogr. 15: 386-394.

Thurman, E.M. (1985). "Organic Geochemistry of Natural Waters". Martinus Nijhoff / Dr. W. Junk Publishers.

Triska, F.J., Kennedy, V.C., Avanzino, R.J. & Reilly, B.N. (1983). In: "Dynamics of Lotic Ecosystems", (T.D. Fontaine III & S.M. Bartell, eds.). Ann Arbor Science.

Triska, F.J., Sedell, J.R., Cromack, K. Jr., Gregory, S.V. & McCorison, F.M. (1984). Ecol Monogr. 54: 119-140.

Valiela, I. & Teal, J.M. (1979). Nature 280: 652-656.

Valiela, I., Howes, B., Howarth, R., Giblin, A., Foreman, K., Teal, J.M. & Hobbie, J.E. (1982). In "Wetlands: Ecology and Mangement. Proc. First Int. Wetlands Conf.", (B.Gopal, R.E. Turner, R.G. Wetzel, & D.F. Whigham, eds.), New Delhi, Sept. 1980.

Vallentyne, J.R. (1970). Can. Res. Development May-June: 36-43, 49.

Van Cleve, K. & Alexander, V. (1981). In " Terrestrial Nitrogen Cycles", (F.E. Clark & T. Rosswall eds.), pp.375-404. Ecol. Bull.(Stockholm) No. 33. SCOPE.

Van Wazer, R. (1973). In "Environmental Phosphorus Handbook", (E. Griffith, A. Beeton, J. Spencer, & D. Mitchell, eds.), pp. 169-178. Wiley, New York.

Vannote, R.L., Minshall, G.W., Cummins, K.W., Sedell, J.R. & Cushing, C.E. (1980). Can. J. Fish. Aquat. Sci. 37: 130-137.

Verstraete, R. (1981). In "Terrestrial Nitrogen Cycles", (F.E. Clark & T. Rosswall, eds.), pp. 565-572. Ecological Bull. (Stockholm) No. 33. SCOPE.

Vogt, K.A., Grier, C.C., Meier, C.E. & Edmonds, R.L. (1982). Ecology 63: 370-380.

Vollenweider, R.A. (1976). Mem. 1st. Ital. Idrobiol. 33: 53-83.

Walker, T.W. & Adams, A.F.R. (1958). Soil Science 87: 1-10.

Ward, A.K. Dahm, C.N. & Cummins, K.W. (1985). J. Phycol. 21: 223-227.

Wallace, A., Romney, E.M. & Hunter, R.B. (1978). In "Nitrogen in Desert Ecosystems", (N.E. West & J.J. Skujins, eds.), pp. 207-218. (US/IBP Synthesis Ser. 9), Dowden, Hutchinson & Ross, Stroudsburg, Penn.

Westin, F.C. & Buntley, G.J. (1967). Soil Sci. Soc. Am. Proc. 31: 521-528.

Wetzel, R.G. (1983). "Limnology". Saunders College Pub.

Wheeler, P.A. & Kirchman, D.L. (1986). Limnol. Oceanogr. 31: 998-1009.

Whitford, W.G., Meentemeyer, V., Seasted, T.R., Cromack, K. Jr., Crossley, D.A., Santos, P., Todd, R.L. & Wade, J.B. (1981). Ecology 62: 275-277.

Whittaker, R.H. (1975). "Communities and Ecosystems". MacMillan.

Woodmansee, R.G., Vallis, I. & Mott, J.J. (1981). In "Terrestrial Nitrogen Cycles", (F.E. Clark & R. Rosswall, eds.), pp. 443-462. Ecol. Bull. (Stockholm) No. 33. SCOPE.

Woodwell, G.M. (1978). Sci. Am. 238: 34-43.

Zlotin, R.I. & Khodashova, K.S. (1980). "The Role of Animals in Biological Cycling of Forest-Steppe Ecosystems", Dowden, Hutchinson and Ross Inc., Stroudsburg, Penn.

The Genetic Basis of Changes in Host Range or Habitat

Fred Gould
Department of Entomology
840 Methodist Road, Unit 1
North Carolina State University
Raleigh, North Carolina 27607
Tel: (919) 737-2638

and

Adrianne Massey
North Carolina Museum of Life and Science
P. O. Box 15190
Durham, North Carolina 27704
Tel: (919) 477-0431

March 1987

The Genetic Basis of Changes in Host Range or Habitat

Introduction

The abundance and distribution of a species is limited by its ability to cope with the abiotic and biotic characteristics of its environment. The environment challenges; the species adapts over time, through the process of organic evolution. These adaptations, which may by physiological, morphological, or behavioral in nature, provide the coping mechanism, but, ultimately, it is the genes of organisms that dictate how well they are suited to their environment. Therefore, genes must provide the primary focus for any discussion of the limitations on the abundance and distribution of species.

Of primary concern is the nature of the genetic information responsible for limiting species' adaptedness to their environments. First, we must assess whether these limits can be broadened by simple genetic changes or require profound reorganization of the genome. That is, could single gene (monogenic) changes lead to alterations in traits that significantly influence the abundance and distribution of a species?

Because the relationship between specific gene substitutions and changes in an organism's phenotype is not always straightforward, determination of the number of genes that contribute to the expression of a characteristic is only the first step in assessing the nature of the genetic information responsible for adaptation. At issue is the manner

in which genes interact with each other. Often a gene's effect upon the expression of a trait depends upon the other genes within the genome, the "genetic environment" of that gene. The joint effect of two or more genes often differs from the sum of the effects of those same genes taken separately (epistasis). Finally, most genes have more than one effect on the organism (pleiotropy). Therefore, changing one gene will more than likely affect more traits than the intended one.

To illustrate how knowledge of the nature of the genetic information responsible for a given adaptation is crucial to predicting the ecological ramifications of introducing genetically engineered organisms to the environment, we provide the following example. The underlying genetic basis of salinity tolerance in plants is polygenic (more than one gene is involved in the trait) and involves epistatic interactions of genes. Thus, there is little reason to believe that a single gene, incorporated into the plant because it codes for some unrelated biological characteristic, would dramatically alter salinity tolerance and thus broaden the range of that plant.

Although an understanding of the genetic basis of adaptation is of fundamental importance in determining what limits the abundance and distribution of a species, a characterization of the precise nature of the adaptation in question may also guide bioengineers in the prudent choice of projects. Understanding the details of the morphological, physiological, and behavioral traits that might dramatically alter a

species' adaptedness to the environment, even when the genetic basis is unknown, could help us predict whether transfer of genes coding for a specific trait would likely change a species' limits.

For example, it has been suggested that genes providing heavy metal tolerance could be incorporated into marine plants for marine pollution control (Vournakis, 1984). Although the genetic basis for this ability is not totally understood, the adaptation would be biochemical in nature. Proteins that have a high affinity for mercury, cadmium, zinc, and copper are known to exist. Genes that code for these proteins could be transferred into marine plants and convert these plants into heavy metal scavengers.

Yet, because we have a handle on the precise nature of this trait, ecologists would add some cautionary notes to what appears to be a panacea for pollution. Their argument would be as follows: The abundance and distribution of many plants is limited by herbivores. Toxins produced by some plants lower the level of herbivory and allow those plants to grow faster and produce more offspring than plants lacking those toxins (Jones, 1966, 1968, 1972, 1973). Therefore, plants with heavy metal binding capability may be viewed as toxic by herbivores, and, if their abundance and distribution is usually held in check by marine herbivores, may outcompete other marine plants. Or, on the contrary, if the plant is a preferred food item and is not avoided by herbivores, heavy metals may enter the food chain. Finally, as is the

case with all gene transfers into plants, we must be concerned with the movement of that gene to plant species other than the intended recipient (see discussion of allopolyploidy below).

Thus, if the need for a heavy metal-scavenging plant were crucial, our knowledge of the biochemical basis of this trait, buttressed by ecological information, would allow bioengineers to anticipate potential ecological problems and select appropriate plant species. In this case, the wise choice would be a plant species that is not limited by herbivory, nor is a favored food of many species, and one that is unlikely to transfer genes interspecifically.

When we lack an understanding of the detailed genetic basis of environmental adaptation, and/or its morphological/physiological/ behavioral expression, useful information may still be extracted from studies that simply document geographical patterns of genetic variation in adaptations. If, for example, we find that a plant genotype surviving in an environment that is adverse to its conspecific relatives is rarely found in adjoining, 'favorable' habitats, a tradeoff for surviving in the adverse environment probably exists. Therefore, interspecific transfer of the trait that allows adaptation to adverse environments is likely to ecologically handicap the recipient organism in some other ways. If we find that variation for a trait is continuous (e.g., individuals can be

found that express a continuum of tolerance for heat stress), we can assume that expression of the trait is not controlled by a group of highly interdependent gene products (epistatic effects).

We have outlined three types of information regarding the limits of environmental adaptation that would be useful in assessing the risks involved in releasing genetically engineered organisms. These are the 1) genetic basis of the adaptation, 2) physiological, morphological and/or behavioral nature of the adaptation, and 3) pattern of naturally occurring genetic variation for the adaptation. Unfortunately, rarely are we blessed with information on each of these for a given trait. In the next section of this paper, we will offer a general review of what is known of these three aspects of adaptation to the environment. It is not our intent to present an exhaustive review of the available literature. Instead, we will attempt to outline a few informative and representative cases, while citing general references that allow the interested reader access to a wider variety of studies in specific areas.

Heretofore, our discussion of the limits to the abundance and distribution of organisms has been described vaguely as "limits in adaptedness." To be more specific, we will define these limits in terms of habitat and/or host range of a species. One can easily see that changes in patterns of habitat use and/or available hosts will likely change the abundance and distribution of a species. We will treat the genetics of change in habitat use and change in host range separately.

By restricting change in habitat use to changes that directly affect an organism's adaptedness to its abiotic environment (e.g., temperature, rainfall, salinity), we need only consider the genetics of one species. When dealing with change in host range, two genetic systems are involved, such as a predator and its prey, or an herbivore and its host plant. An herbivore's host range can broaden because it genetically adapts to a toxin produced by a plant species, or because that plant changes genetically and no longer produces the toxin. In addressing the potential for humans to alter host range, we must examine the genetics of all the interacting species. If, as a hypothetical example, we find that there is no way that genetic engineers could change an insect parasitoid's parasitic ability, this does not mean we must throw up our hands in resignation. Genetic engineers have the option of tinkering with the inherent susceptibility of a potential host of the parasitoid. Thus, by altering the host and not the parasitoid, the host range of the parasitoid is changed.

Such flexibility is not the straightforward blessing it may seem, however. In any ecological system where species influence each other's abundance and distribution, a simple genetic change in one species has the potential for affecting many members of the community. If one plant species gains a gene for immunity to one serious pathogen, that plant might grow faster and thus increase in its competitive ability. This could lead to elimination of some other plant species from the community.

Keeler and Dobson (in another OTA report) have addressed these problems under the heading of 'cascading effects.' Although our concern here will be the type of genetic changes engineered by humans that could change the outcome of one-to-one species interactions, community-wide effects are to be expected and must be kept in mind.

In the sections that follow this we shall cover the empirical literature on habitat/host range characteristics in the following order:

1. Habitat Related Adaptations
    - A. Microbes
    - B. Plants
    - C. Animals
2. Host Range Related Adaptations
    - A. Plant hosts
        1) microbes
        2) herbivorous animals
    - B. Animal Hosts
        1) microbes
        2) parasitic animals

For each of these topics we will discuss the three relevant factors discussed above (genetic mechanism, nature of the adaptation, pattern of genetic variation). After covering the empirical literature related to these areas, we shall attempt to draw some conclusions about the types of engineered, gene transfers that are more likely to have direct effects on host range or habitat use, and therefore, species abundance and distribution. We shall also add a section that deals with the important question of how indigenous organisms (whether pests or beneficials) are likely to genetically respond to an engineered organism that affects their fitness.

1. Habitat Related Adaptations

A. Microbes

The literature on genetically controlled differences between microbial strains in traits related to habitat adaptation is enormous (Hayes, 1968). Much of this information was accumulated in the pursuit of a detailed understanding of the bacterial genome, so little emphasis seemed to have been placed on the real world ecological context of the phenotypic differences between strains caused by single DNA base pair mutations. On the other end of the spectrum of microbial investigations are ecological and physiological investigations of microbial survival in "extreme" habitats (e.g., Thayer, 1968). These studies have focused mainly on differences between bacterial species in tolerance of environmental conditions, although a number of good comparisons of strains are available.

Briding the gap between detailed genetics and evolutionary ecology are studies of bacterial adaptation to antibiotics. In their natural environmental context, antibiotics mediate intra- and interspecific interactions, thus adaptations to them would belong in a different section of this report. However, the widespread production and use of these toxins by humans in a fashion that decouples them from the genetic system of their original producer has caused their ecological activity to resemble that of abiotic environmental factors. In this context, an

understanding of microbial adaptation to these extreme environments is instructive in predicting future adaptation to humanly manipulated environments.

1) Physical environment

Microbes have adapted to some of the most physically diverse environments on earth. Some bacterial species have been found growing at temperatures up to 110°C (Brock, 1985), while other species have been found to reproduce at -23°C (Skinner, 1968). Bacterial species have been found that can tolerate other abiotic stresses as diverse as 1000 atmospheres of pressure and 25% salt concentrations.

In terms of genetically determined strain differences, Brock and Brock (1966) found that hot spring bacteria at Yellowstone Park had different temperature optima according to their position along a stream flowing away from the natural heat source. This study seems to imply that within a species there are tradeoffs in terms of adaptation to abiotic environmental factors. Therefore, even though a strain of bacteria with a broad temperature tolerance would have been favored in this unique stream environment, such a strain has not evolved.

Heat stability of enzymes is often associated with the optimal growth temperatures of a microbe (Skinner, 1968). Campbell (1954) found that when a strain of bacteria was cultured at two different temperatures (35 and 55°C), the two cultures differed in the stability of their alpha-amylases at 90°C.

Some of the detailed molecular characteristics that allow for enzyme stability at high temperatures have been investigated (Brock 1985). In at least one case a single amino acid substitution led to a 10°C difference in thermostability. Since survival at high temperatures requires stability of a large number of enzymes, engineering heat sensitive bacteria for high temperature survival is less likely than transferring a gene for a specific function into a heat tolerant species. Currently, efforts are being made in the United States and Germany to catalog the biological characteristics of heat tolerant species (Brock, 1985).

In addition to differences among bacterial species in temperature tolerance, there is evidence that some of their phages have adapted to high temperatures (Saunders and Campbell, 1966). This brings up possible approaches for the transfer and maintenance of specific genes in these thermally tolerant species. The consequences of adding novel biological abilities to this previously, ecologically restricted group has not been addressed.

2) Nutrients

There are hundreds of auxotrophic mutants of bacteria that are incapable of surviving without specific nutrients (Hayes, 1968). These mutants could be valuable in the building of "unarmed" bacterial strains that would have a low probability of survival outside the confines of the

laboratory or commercial fermentation tank. It should be possible to develop strains with multiple deficiencies that are unlikely to all be repaired by single plasmid transfers or back mutations.

Much more is known about deficiencies in nutrient metabolism than is known about the acquisition of enhanced ability of a bacteria to produce a limiting nutrient. Certainly different bacterial species require different nutrient supplementation, so it would seem possible that strains of a specific bacteria could be engineered that would have fewer nutrient requirements. How this would effect the ecological niche of these strains is unknown.

3) Toxins

Microbial adaptation to toxic chemicals was first reported in 1887 (Kossiakoff, referred to in Koch, 1981). Kossiakoff found that Bacillus subtilis had become tolerant to the antiseptics boric acid and mercuric chloride. In the early 1900's, resistance to other toxins such as arsenicals and parafuchsin were reported (see Ehrlich, 1959). Adaptation to the "early" antibiotics as well as the antibiotics in the post-penicillin era did not appear to be a difficult evolutionary hurdle for microbes. Chain et al. (1940) found that penicillin was clinically effective. A year later they reported resistance to it (Abraham et al., 1941). In the 28 years since, we have experienced a proliferation of antibiotics and microbes resistant to these antibiotics (Koch, 1981). Although bacterial resistance has been the major area of concern, drug resistance in other single cell pathogens such as the malarial plasmodium

have also caused concern (Spencer, 1985). This rapid adaptation of some microbes to antibiotics prompted the following sobering, prophetic statement by Lowbury and Ayliffe (1974):

> "We gave good reason to be anxious about the future. It seems likely that in forty years' time a doctor may recall the life-span of antibiotic therapy as a short episode in the long art of medicine; that, at least, might be the outcome for certain bacteria, such as Staphylococcus aureus and the tubercle bacillus, if we do not devise and adopt better ways of arresting the trend towards resistance than those in current use."

Since 1974 there does not seem to have been any new breakthrough in arresting the trend toward resistance, although our understanding of the genetic process involved in microbial adaptation to antibiotics has become increasingly sophisticated (see Mitsuhashi and Hashimoto, 1975; Koch, 1981; Quesnel and Russell, 1983 for reviews). The mechanisms by which bacteria have adapted to antibiotics are quite diverse. They include inactivation, metabolism, change in the site of action, impermeability of the cell envelope (e.g., membranes and secretions), and tolerance of the drug's effects (Quesnel and Russell, 1983).

The genetic material coding for antibiotic resistance has been found associated both with the bacterial genome and with the genetic material of bacterial plasmids (indeed these genes can move from bacterial

chromosomes to the plasmids (Hedges and Jacob, 1974). Plasmid-borne resistance is very common and seems to involve all resistance mechanisms except changes in the site at which the antibiotic acts. Changes in sensitivity of the site of action generally involve small changes in structural proteins of the bacteria, and structural proteins are rarely coded for by plasmids (Koch, 1981).

Two very important characteristics of plasmid-borne resistance are that it can be transferred from one bacterial genus to another (e.g., Ochiai et al., 1959; Clewell, 1981), and a single plasmid can carry genes coding for a number of different types of antibiotic resistance. The phenomenon of transferrable multi-drug resistance was first noted in 1959 in Japan (Ochiai et al., 1959; Akiba et al., 1960; Hayes, 1968) and was subsequently recognized as the explanation for many occurrences of multiple drug resistance (Mitsuhashi and Hashimoto, 1975). More recently, evidence has appeared indicating that resistance factors can sometimes be transferred between bacteria even in the absence of plasmids (Clewell, 1981).

Multiple drug resistance is often the result of plasmids that are carrying genes that code for a number of separate resistance factors (e.g., PCase plasmid, see Kondo et al., 1975), but there are also many well studied resistance factors such as Beta-lactamase, coded for by one gene, that are responsible for resistance to a large class of antibiotics (e.g., Daum et al., 1981). Thus, whether due to gene linkage (on the

plasmid) or because of pleiotropic effects, it is reasonable to expect the selective pressure on a bacterial strain for adaptation to one antibiotic may lead to resistance to a broad array of antibiotics.

Plasmid-borne, multiple resistance sometimes extends to chemicals not generally considered to be antibiotics in the classical sense. Evidence that has accumulated regarding the characteristics of Staphyloccus aureus strains carrying plasmids for penicillin resistance indicate that a number of these are also resistant to heavy metals (Kondo et al., 1975). Studies of the mechanisms promoting metal tolerance in these strains indicate that at least two separate changes in physiology are involved (Kondo et al., 1975). Genetic experiments indicate that a number of genes are involved and that plasmids can be obtained that code for resistance to specific heavy metals, but not to antibiotics. This knowledge is especially important in light of the fact that one proposed use of microbial bioengineering is development of bacteria that can help with pollution problems. It would be unfortunate if a bacteria developed to accumulate heavy metals (or just survive in a heavy metal contaminated environment) carried with it transferrable antibiotic resistance genes.

Another far reaching effect of plasmids coding for antibiotic resistance was discovered by Reynard et al. (1978). They found that Escherichia coli carrying plasmid R100 that conferred multiple drug resistance was more resistant to the effects of serum defenses of rabbits that E. coli lacking the plasmid. Not all multiple drug resistance

plasmids caused this cross-adaptation to rabbit serum defenses, and to further complicate the issue, bacteria containing the R100 plasmid plus an I-like drug resistance plasmid were less resistant to the rabbit serum than strains carrying only R100. These results give us some indication of the possible complexities involved in predicting the effects on the biology of a bacteria of an uncharacterized plasmid.

One straightforward effect of metabolizing antibiotics or producing impermeable membranes would seem to be a cost to efficiency in carrying out other life functions. In many cases it has been shown that competition between strains with and without antibiotic resistance in a benign environment leads to elimination of the resistant strain (e.g., Koch, 1981). This is not, however, the only result. In a few carefully monitored studies, antibiotic resistance in farm animals persisted long after the use of antibiotics was halted (e.g., Richmond, 1977; Koch, 1981).

Perhaps the most important question, and the most difficult to answer regarding cost of resistance, is whether when we measure a loss of resistant plasmids from a population of bacteria following cessation of antibiotic treatment, is the plasmid lost completely? If, instead of becoming extinct, the plasmid is able to survive at very low frequency (e.g., 10^{-10}), reintroduction of an antibiotic could cause rapid reappearance of resistance.

B. Plants

At present, studies on the genetics of wild plant populations are of two types: 1) ecological studies of plant populations that demonstrate adaptive genetic differences both between and within populations, and 2) genetic studies on the precise mechanism of inheritance of phenotypic traits. Unfortunately for our purposes, these studies have proceeded not in series but in parallel. For any given wild plant species where naturally occurring, genetically based adaptations have been shown to exist, the mechanism of inheritance of these adaptations is unknown. Where genetic mechanisms of traits are known, adaptive genetic variation in nature is not. Therefore, we must discuss these two types of studies separately.

Genetic Variation in Wild Plant Populations

Variation Between Populations

For almost 100 years, beginning with the investigations of Kerner (1891), botanists have been amassing data on the extent to which different populations of a given plant species differ. The existence of an immense amount of difference between populations is now an established fact (for reviews see Hamrick, 1979; Bradshaw, 1984).

In biological systems such variation may be phenotypic and induced by the immediate environment, or it may have a genetic basis and be the result of long-term environmental selective forces. Because only

genetically based variation is a factor in evolution, of primary importance is the degree to which the observed differences between plant populations can be explained by genetic differences. Through use of the "common garden" technique where plants from different populations are transplanted to a shared site, thus removing the direct effects of differing environments, underlying genetic differences between populations have been revealed for an extraordinary array of wild plants.

For evolutionary biologists, the next crucial question is whether the observed genetic differences are adaptive, that is, are the result of natural selection or are simply present for historical reasons. This issue has been addressed for a variety of wild plant species using reciprocal transplant experiments. Genetically different, conspecific plants from different populations are planted in both their natural environment and the other population's natural environment. Measures of survival and reproductive success are then assessed. The genetic differences are considered adaptive if the plants do better in the "home" than their "away" environments. Genetic adaptation to abiotic, physical environmental factors has been shown for a variety of plant species. Some of the abiotic factors to which plants have been shown to adapt include a wide variety of soil factors, level of submergence in aquatic populations, wind exposure, temperature, irradiance, level of heavy metal

pollution, and more recently, herbicides. To date, 30 annual weed species in 18 different genera have genetically adapted to the triazine group of herbicides by becoming resistant to them (LeBaron and Gressel, 1982).

Adaptive genetic change to abiotic conditions may occur over a brief period of time. Studies of adaptation in plants to various edaphic conditions illustrate such rapid change. For example, Anthoxanthum odoratum showed genetically based physiological adaptation to the addition of lime to the soil within 7 years (Snaydon, 1970). Rapid adaptation has also been demonstrated for tolerance to soil copper (Bradshaw and McNeilly, 1981).

The nature of these adaptations may be morphological, biochemical, physiological, or behavioral. Morphological adaptation may be limited as in Ranunculus aquatilis, Lolium perenne, and Leptospermum flavescens where only leaf shape and size exhibit differentiation, or very broad as in the case of Agrostis where 8 morphological attributes were found to differ between populations adapted to various habitats (Cook and Johnson, 1968; Morrison and Myerscough, 1982). Adaptation to level of irradiance in Plantago has been ascribed to differences in photosynthetic ability that result from high rates of enzymatic activity and differential CO_2 diffusion capacity (Teramura and Strain, 1979). A variety of plant species have adapted to varying levels of soil acidity (example, Trifolium repens, A. odoratum, Festucs spp.). This adaptation is based

upon physiological differences in the ability to uptake, transport, and utilize elements such as calcium, phosphate, potassium, and iron (Snaydon and Bradshaw, 1961; Snaydon, 1962; Snaydon and Davies, 1982). Finally, date of flowering, a behavioral trait, varies in populations differentially adapted to various habitats (Snaydon and Davies, 1976).

In addition to adapting to the physical environment, organisms may also adapt to the biotic factors within their environments. Other living organisms may exert strong selective pressures that result in genetic differentiation between populations through the forces of competition and predation/parasitism (for reviews see Dirzo, 1984; Turkington and Aarssen, 1984). For example, grazing intensity results in variation in time of flowering in populations of L. perenne (Breese and Charles, 1962). Again, the time scale over which adaptation may occur is quite brief. Rye grass (Lolium) adapted to lax grazing within four months of application of the grazing regime (Brougham and Harris, 1967).

Variation Within Populations

In a sense, the extraordinary extent to which plant populations were found to differ genetically between themselves is not surprising. Populations that inhabit different environments (and therefore are exposed to different biotic and abiotic selective pressures) and that do not exchange genes are expected to diverge genetically over time. What

was surprising, but is now considered commonplace, is the marked degree of genetic differentiation that isests WITHIN populations of plants where free gene flow is occurring (for review see Antonovics, 1984).

Such variation indicates that selective pressures must be 1) strong enough to swamp gene exchange that would result in a genetically homogenous population, and 2) operate on a very local scale. For example, Veronica peregrina individuals located at the edge of temporary pools differ genetically from individuals located 2 m away in the center of the pools. Selective pressures that may have resulted in this differentiation include biotic factors such as competition and predation and abiotic factors such as moisture and temperature (Linhart, 1974). Even more striking are the genetic differences between individuals in a population of Anthoxanthum. Significant differences in a number of behavioral and morphological attributes were found between individuals living only 0.1 m apart (Snaydon and Davies, 1976).

Genetic Adaptation and Ecological Range

To what extent, or in what fashion, is the extreme genetic differentiation we find in plants related to the ecological range of individual species, that is, its occurrence and overall survival in a range of habitats? Two factors related to genetic variation appear to affect the range of habitats a species may inhabit: the degree of

existing genetic variation in a species that allows for adaptation to new habitats (genetic flexibility) and the presence of plants that are already adapted to that habitat (superior competitors).

Genetic Flexibility and Ecological Range

Different species do not have the same evolutionary abilities, and these limits to evolutionary differentiation will affect whether or not a species can exist in a certain habitat. For example, it has been demonstrated experimentally that the ability to evolve metal tolerance (and therefore to extend its range to metal contaminated soils) is limited in certain species and that this limitation is based upon the lack of necessary genetic variation (Gartside and McNeilly, 1974).

Superior Competitors

Genetic adaptation to biotic and abiotic factors in one environment may lead to increased survival and reproductive success in that environment, but does not guarantee success in a second environment where conspecific individuals adapted to that environment exist. Indeed, it appears that plant species are composed of populations that will not survive in each other's habitats. For example, plants adapted to metal-laden soils may survive well in normal soils in the absence of competitors. In the presence of competitors, however, the survival of metal-tolerant populations relative to normal populations is much lower (Hickey and McNeilly, 1975). Similar results have been demonstrated for

natural populations of Achillea (Clausen et al., 1948), Trifolium (Snaydon, 1962), Anthoxanthum (Davies and Snaydon, 1976), Agrostis and Festuca (Smith and Bradshaw, 1979), and in cultivated populations of barley (Harlan and Martini, 1938) and rice (Jennings and de Jesus, 1968). In general, the relative fitness of alien species is one-half (0.5) that of resident species (range 0.01-0.95) (Levin, 1984).

In summary, because the distributions of most plant species appear to be stable (Bradshaw, 1984), we must assume that the presence of superior competitors and the lack of appropriate genetic variability are preventing significant range expansions in plants. This, of course, refers to existing conditions. Introduction of genetic variatility to a plant species through bioengineering may lead to a range expansion for the intended recipient plant, or for other unintended plant species due to the ability of plants to transfer genes between species.

Allopolyploidy

For most animals, the designation of "species" carries with it the concept of reproductive isolation. That is, one animal species cannot breed and cannot exchange genes with another species. This is not true of plants. When two distinct species hybridize and the resulting offspring is fertile, this offspring is usually reproductively isolated from each parental species, and thus a new species is immediately formed. The new species is termed an alloploid and the phenomenon of possessing chromosomes from two sources, allopolyploidy. Stebbins (1971) suggests

that 35% of the existing flowering plant species have evolved through allopolyploidy, a particularly common characteristic of many crops such as wheat, oats, cotton, tobacco, and forage grasses.

Most instances of allopolyploidy involve the interbreeding of two species within the same genus, but gene exchange may also occur between plants in different genera. For example, wheat was derived from the hybridization of a species in the Triticum genus and one in the Aegilops genus.

As stated above, most alloploids are genetically isolated from their parental species. However, gene flow between the alloploid and the parental species does occur, usually in the direction of parent to alloploid (Briggs and Walters, 1984). Levin (1978), however, recently documented cases of gene flow from the alloploid to the parental species in Solanum, Viola, and Papaver.

In summary, there is no guarantee that genes placed in a given plant species will stay in that plant species.

Basic Genetic Mechanisms in Plants

The genetic mechanism underlying variation may be quite diverse. In some cases the differences may be due to single locus differences; in others, many genes may be involved. We know almost nothing of the genetic bases of the adaptations described above. One of the few adaptations of which we know something of the genetic basis is herbicide resistance. Weed species possess a wide variety of inheritance modes for

herbicide resistance, both polygenic and monogenic (Cormstock and Anderson, 1968; Schooler et al., 1972; Warwick and Black, 1980; Scott and Putwain, 1981).

Although we know little of the genetic bases of the adaptations described above, we do know that in plants morphological variation may be ascribed to single gene or polygenic systems.

In a wide variety of grasses, both cultivated and wild, an albino seedling form is not uncommon. This albino condition is due in most cases to a single recessive gene (Crumpacker, 1967). In sweet peas (Lathyrus odoratus), a single gene mutation produces dwarf plants in the homozygous state and another single gene affects flower color (Crane and Lawrence, 1934). When a character is affected by a number of genes it is said to be polygenic. Examples of plant characters that are known to be under polygenic control are corolla length in Nicotiana longiflora (East, 1916), kernel color in wheat, Triticum aestivum (Nilsson-Ehle, 1909), and fruit shape in squash, Cucurbita pepo (Sinnott and Hammond, 1930).

C. Animals

The literature on behavioral and physiological mechanisms by which animal species and populations within species adapt to their habitats has blossomed in the past 20 years. This is largely due to the development of the fields of behavioral ecology and evolutionary ecology. The type of traits investigated range from timing of reproduction to thermoregulatory behavior to sex ratio to nutrient requirements. A

number of textbooks can be consulted to gain a general perspective on research addressing the question of within species, habitat-related, genetic diversity (e.g., Futuyma, 1979).

Most of this work has been concerned with the adaptive significance of specific inherited traits, while only a small percentage of the studies have focused on the details of how these traits are inherited. One important factor limiting the study of inheritance is the long generation time of many animal species. Thus, aside from studies of domesticated animals, most of the detailed genetic studies have focused on short-lived organisms such as insects (Huettel, 1987).

The work on domesticated animals has mostly focused on fine tuning these animals to their specialized environments. Animal breeding of this sort has uncovered a huge amount of variance in specific traits. One only needs to look at the differences in the habitat requirements of wild and domesticated turkeys to get a feeling for this variation. Most of the variation that animal breeders have worked with appears to have a polygenic basis (Falconer, 1981).

Research on the detailed inheritance of insect, habitat-related adaptations has focused on two general topics: adaptation to seasonal environments and adaptation to human alterations of the habitats of insects. We will concentrate on results of this work since they

illustrate the type of changes in the animal genome needed for adaptation to geographical and temporal changes in habitat characteristics and because insects may be one of the targets of genetic engineering.

Since most insects are ectotherms (i.e., cold-blooded), they must adapt to the thermal qualities of their environments. In temperate climates this often means coping with temperatures below 0°C and above 35°C. Insects have developed a number of strategies for coping with these extreme seasonal temperatures, the most common being an arrested state of growth called diapause. Initiating or terminating diapause at the wrong time can be maladaptive, and since the optimal timing differs geographically, insect populations within a species are often found to differ in their diapause characteristics (Tauber et al., 1985). The detailed genetics of sensitivity to cues used to initiate and to terminate diapause has been studied in more than 10 species of insects (Tauber et al., 1985). Crosses between strains that do and do not diapause indicate that one or a few genes may be involved. In the case of the European corn borer where some strains automatically go into diapause after each generation, while others go only into diapause in response to specific environmental cues, the difference between strains appears to be coded for by a single sex-linked gene (Showers et al., 1974). Obviously, adaptation to some aspects of the thermal environment require only superficial genetic changes.

Humans have intentionally and unintentionally altered the habitats

of insects in many ways. While many insect species have responded by becoming extinct, others have adapted to these changes rather rapidly. The best studied cases of insect adaptation to intentional alterations involve the evolution of insecticide resistance. The evolution of cryptic melanism in response to side effects of industrialization stands as the most carefully documented case of adaptation to unintended habitat alterations.

Cryptic Melanism

One way in which many animals avoid predation is to blend into the patterns and colors of their environment so that visual detection by predators is difficult, if not impossible. Insects are no exception. Wing coloration and patterns in nocturnal moths often match that of their daytime resting places, such as the bark of trees. Previous to the industrialization of Europe, the bark of many trees was covered with lichens and light in color. Following industrialization, soot from factory chimneys was deposited on tree bark, killing the lichens and darkening the tree bark. Formerly cryptic moths became readily detected by avian predators (Kettlewell, 1973). Entomologists and geneticists, primarily in England, observed how in a very short period of time many moths adapted to this change in habitat by evolving dark wing pigmentation. Kettlewell (1973) has summarized the ecological and genetic literature on this topic. One fact that stands out in the research on all of the species studied is that single alleles, usually

with dominant expression, were the major factor in adaptation. Kettlewell (1973) presents a number of hypotheses to explain the predominance of these single gene changes, but what is important for this report is the obvious fact that a single gene change can have major effects on habitat adaptation. Studies of bird predation of moths that were at rest on matching and nonmatching tree bark indicated up to a 10-fold difference in survival.

Because of the detailed genetic crosses conducted on a few moth species it has been possible to show that the alleles coding for melanism in different populations of a single species can be of different origin, and most importantly, that expression of these different alleles depends on the population of moths into which it is placed (Kettlewell, 1973). If such a phenomenon is common, it may be difficult to generalize about the effect of incorporating one gene into a species, based on laboratory studies in which a gene is engineered into one animal colony.

Insecticide Resistance

It is useful to compare insect adaptation to the above, physical change in a habitat and insect adaptation to the biochemical habitat change brought about by the use of pesticides. While it may not be surprising to some scientists that superficial genetic changes could adapt organisms to a change in habitat coloration, adaptation to chemicals that disrupt nerve function or hormonal regulation must be seen as profound habitat alterations.

The immense literature on the chemistry, toxicology, and environmental effects of insecticides reflect society's concern with the far-reaching effects that these chemicals can have on organisms. Given the incredibly small quantities of these compounds that can kill organisms ranging from humans to fungi, it is amazing to note how rapidly insects, the targets for these chemicals, adapt to their presence. To date at least 447 species of insects and mites have adapted to at least one insecticide, and 23 species have already adapted to the newly registered pyrethroid insecticides (Georghiou, 1986). Most of the resistance to insecticides is due to biochemical changes in the insects (Plapp, 1976, 1986), although an increasing number of reports of behavioral changes that confer resistance are being reported (Gould, 1984).

Amost as surprising as the rapidity with which insects have adapted to insecticides is the fact that in the majority of cases of field resistance to insecticides, one or a few genes control at least a major portion of the resistance (Plapp, 1986; Roush and Croft, 1986). These single genes have been associated with changes in the site at which the insecticides act (e.g., altered acetyl-cholinesterases), increased levels of xenobiotic metabolizing enzymes (e.g., mixed function oxidases, glutathion transferases), and decreased penetration of the insect's cuticle (Plapp, 1976).

As with antibiotic resistance, single gene changes in the insects may cause resistance to more than one toxic compound, and these compounds may be unrelated structurally or in mode of action (Plapp, 1976). Again, as in the case of bacteria, there are sometimes physiological costs associated with these single gene changes, but in many cases these costs are small or nonexistent (Roush and Croft, 1986). Case studies have shown that once resistance has developed in a population, removal of the insecticide does not always lead to reversion of the population to susceptibility (Keiding, 1986). This indicates a lack of ecological costs involved in maintaining the resistance mechanism.

The genetic background in which a major resistance gene is found can have an effect on its cost to the organism. McKenzie et al. (1982) found that a major gene for resistance in a population of the sheep blowfly did not affect that population's fitness. However, when that gene was backcrossed into another blowfly population, it did decrease fitness.

2) Host Range Related Adaptations

a) Plant hosts

Microbes. Microbes that decrease the fitness of their hosts are commonly referred to as pathogens. Most of our understanding of the genetic basis for host range limitation in plant pathogens comes from the agricultural literature, although some exemplary studies have been conducted on pathogens of natural plant populations. There is an impressive degree of variation among plant pathogens in the breadth of

their host range. Some pathogen species can infect plants in a number of different families, while others can only infect certain genotypes of a single plant species. In some cases of specialized pathogens, the ecological/physiological interactions between the host plant and pathogen are so intricate that it is hard to imagine a single gene change in the pathogen allowing it to broaden its host range to include plants of other genera. For example, the plant Silene alba is the host of a specialized fungal pathogen. This systemic pathogen causes alterations in the physiology of S. alba that allows production of spores in the plant's flowers. These flowers produce more nectar than uninfected flowers and are visited by pollinators that transmit the spores to flowers of other S. alba plants (Alexander, in prep.). The exact nature of the systemic physiological interactions between these two species is not completely understood, but is likely to be complex and finely tuned. Although there is evidence for variation among genotypes of the fungus in the range of S. alba genotypes they can infect (Alexander, 1987), the existence of a single gene that could expand the fungus' host range to include other plant genera is highly doubtful.

If we move from this extreme case of specialization to some of the more common pathogen/host associations found in agriculture, it becomes apparent that single gene changes can have important effects on host range. Indeed, a number of authors have argued that single gene changes have played a prominent role in the evolution of pathogen host ranges

(e.g., Flor 1942, 1971; Nelson 1979). Given the potential negative impact of plant pathogens on modern agriculture, the literature on the genetics and physiology of host range limitation is enormous. A number of detailed reviews of this area are available in a five-volume series entitled, Plant Pathology: An Advanced Treatise (see volumes 4 and 5, Horsfall and Cowling, 1979, 1980). We will present some cases that establish the range of effects that single gene changes in pathogens or their hosts could have on the host range of the pathogen.

Although plants are not considered to have an immune system comparable to higher animals, more and more evidence is accumulating that suggests that a plant's recognition of a pathogen is an important factor in limiting infection (e.g., Horsfall and Cowling, 1980; Ouchi, 1983). In some cases a single compound produced by a pathogen enables a plant to recognize the pathogen and begin to produce defenses (Ouchi, 1983). In other cases, a single compound may suppress a plant's pathogen-triggered defense system (Ouchi, 1983) and thus permit infection.

The ecological effects of change in these two types of single compounds could be somewhat different. For example, the suppressors in Mycosphaerella pinodes (pea blight fungus) enhanced the ability of another pathogen, Alternaria alternata to infect a number of plant species (Ouchi, 1983). Thus, transfer of a single gene coding for production of a suppressor could affect the parasitic ability of a fungus on more than one host. Furthermore, infection of plants with pathogens

producing suppressor factors could limit the plant's ability to ward off infection by fungus species that were previously nonpathogenic to it (Ouchi, 1983).

In the case of elicitors, gene deletions or substitutions may affect host range. A number of studies have assessed the roles of specific pathogen-produced compounds in eliciting defensive responses in the host. Although the change in virulence of a pathogen due to a single gene substitution or deletion has never been tied directly to a change it caused in production of an elicitor compound, there are some theoretical reasons to suspect such causation (but see Sequeira, 1980). We could not find studies indicating that the same elicitor caused defensive responses in more than one host species. If elicitors are host-specific, their removal due to engineered deletions may have tightly controlled effects. The important point to be gained from studies of both elicitors and suppressors is that the host range of a pathogen may be limited, not by some complex, interactive, biochemical pathways, but simply by the possession of a single compound that genetic engineers could potentially eliminate or insert by a single gene transfer.

In contrast to host range changes that could result from evading plant defensive responses, some pathogens have the capacity to unarm a plant by metabolizing its defensive compounds. Metabolism of the toxin pisatin by Netria haematococca (the perfect stage of Fusarium solani) appears to allow this fungus to survive on pea plants. Tegtmeier and

VanEtten (1982) found a good correlation between ability to demethylate pisatin and a fungal strain's ability to infect peas. Mixed function oxidase enzymes, which generally metabolize a wide array of substrates, have been implicated in this metabolic process (Matthews and VanEtten, 1983). Although other work by Denny and VanEtten (1982) indicates that metabolic capacity is not the only factor involved in host range evolution in these fungi, it is important to note that more detailed experiments with mixed function oxidases in mammals and insects indicate that there are multiple forms of these enzymes with varying substrate affinity (see next section). A change in one structural gene could therefore change the ability of a fungus to metabolize a host toxin. One goal of genetic engineers is to produce microbes with special metabolic capabilities. If this engineering involves changes in enzyme systems such as mixed function oxidases that have broad substrate specificity, there may be reason for some concern, even if the microbes were not initially pathogens (also see Lenski and Istock's report to OTA).

In the cases cited above, we have some understanding of mechanisms that could alter host range. There is a much larger body of research that deals with the inheritance of host range altering genes where the underlying physiological mechanism is not understood. This extensive literature has been reviewed (Lamberti et al., 1983) and indicates that plant resistance to pathogens may be controlled either polygenically or monogenically. From the pathogen side, the data also reveal many cases

where single gene changes can have an all or none effect on pathogenic ability. Not only can a single gene change affect a pathogen's ability to infect different genotypes within a plant species, such changes can affect a pathogen's ability to infect two or more plant species. Whatever the mechanism coded for by plant and pathogen genes, it is obvious that host range limimting factors are superficial at the genetic level. None of the geographic patterns of variation in pathogen host range or genetic crosses implicate epistatically interacting genes in pathogenicity.

If it is so simple for a pathogen to change host range, we should expect little host constancy even within a single pathogen species. At least in some cases this is true. For example, Fusarium solani has some forms which attack plants in the cucurbit family and other forms that attack legumes. Plant pathologists have tried to carefully assess the physiological and ecological cost to a pathogen strain of adding extra hosts to its range. In a few cases, significant costs, measured as decreases in maximum reproductive rate, have been found (Leonard, 1986). In most cases, however, these costs are less than 10% or are nonexistent (Parlevliet, 1983).

A. Plant Hosts

2) Herbivorous animals

More is known about the genetics of herbivory in arthropods than is known about all other herbivorous animals combined.. This is most likely

due to the economic importance of this taxon and the ease with which some of its members can be cultured. The study of genetics of pathogen/plant interactions and that of arthropod/plant interactions has proceeded within separate academic disciplines (entomology and plant pathology). In general plant pathologists have placed more emphasis on this area than entomologists who have focused on the genetics of insecticide resistance. Entomologists have recently put more effort into this area and have been accompanied by evolutionary biologists, who find herbivorous insects to be good subjects for ecological/genetic studies. It is of interest to note the general similarities and differences in the conclusions drawn about genetics of the interactions between these two taxa and their host plants.

As with plant pathogens, we find some species of arthropods that are incredibly specialized and other species that can feed on everything from dicots to monocots to gymnosperms. In the case of certain very specialized insects (e.g., fig wasps [Wiebes, 1979] and yucca moths), it is hard to imagine that a one-gene change would dramatically alter their intricately woven interactions with their host plants. On the other hand, there are a good number of very specialized insect species whose sibling species feed on a broad array of monocots and dicots. For example, Heliothis subflexa specializes on one genus of angiosperm, Physalis, while Heliothis virescens feeds on plants in over 14 families. These two insect species are so similar that they can be crossed with

each other and produce some fertile offspring (Laster et al., 1976). H. subflexa is a rare species, while H. virescens is one of the most important crop pests in the western hemisphere. Obviously the genetic factors limiting host range in these species are not as profound as one might have intuitively expected. The hybrids of these two species are intermediate in host range (Laster, 1982), thus in this case, complex epistatic genetic interactions (coadapted gene complexes) do not seem to predominate in the genetics of host range limitations. A similar type of genetic control on the host ranges of two sibling dipteran herbivore species has been reported (Carson and Ohta, 1981), so the case of Heliothis is probably not an exception to the rule.

One major difference between herbivorous animals and pathogens is that in general pathogens are passively transported between hosts, while most herbivorous animals (including aphids and mites) exercise a degree of control in choosing their hosts. Plant characteristics that limit pathogen host range must act at the physical or physiological level, while herbivorous animals may also be limited by plant traits that affect their behavior.

Economically important plant strains that are resistant to insect attack have been analyzed both regarding the mechanisms and genetics of resistance. Table 1 (from Gould, 1983) presents a summary of these studies. It can be seen from this summary that variation in physiological (antibiosis) and behavior modifying traits (nonpreference)

is common. Both monogenic and polygenic control of this variation has been found. In only one study was epistatic interaction of genes found to have an overriding effect on expression of resistance (Ferry and Kennedy, in press). Polygenic control may be more common than is evident from this table since the studies reported in the literature may be biased in favor of exploring simple traits (see Gould, 1983). Nonetheless, single genes certainly can be important in a plant's ability to limit herbivore damage. In some cases, these single genes may limit more than one herbivorous species (e.g., Lukefahr and Martin, 1966).

One important fact that has arisen from these studies of the genetics of plant resistance to herbivores has been that a gene coding for synthesis of a compound that limits one herbivore may actually increase the plant's susceptibility to another herbivore. A recessive gene that limits production of the chemical, cucurbitacin, in cucumbers makes these plants unattractive to cucumber beetles, while making the same plants susceptible to spider mite damage (Da Costa and Jones, 1971). It behooves genetic engineers to keep this kind of potential side effects of gene transfers in mind.

Studies of genetic variation in host use by populations of herbivorous species indicate that such genetic variation is common. Table 2 (from Gould, 1983) summarizes oure knowledge about this genetic variation. Unfortunately, there are very few cases in which the details of genetic control of these traits or the mechanisms involved have been carefully studied. In two cases where detailed genetic crosses were

carried out, it is apparent that single genes have very strong effects in determining host range (Hessian fly and Sitophilus oryzae). It is difficult to assess whether these results will be generalizable.

Since, for the most part, we lack direct genetic information on within-species variation in herbivore host range, it is worth drawing some inferences about genetics of host-range limitation from studies that have examined the plant characteristics that inhibit survival of herbivores. Physical defenses such as thorns and sticky hairs abound in the plant kingdom. Small insects and mites do not seem to have many ways of circumventing the effects of these traits. When they do, it appears to involve complex adaptations (e.g., Rathke and Poole, 1975). In some cases, hirsuteness (hairiness) is governed by single genes and could plausibly be transferred via genetic engineering. However, the herbivore limiting traits that are likely to be most easily transferred by genetic engineers are chemical in nature.

Plants abound with a wide array of complex organic molecules, termed secondary plant compounds by plant physologists. Many of these compounds are toxic to herbivores and/or modify the herbivores' behavior. Recent studies have documented a number of cases in which strains of an insect or mite have overcome the effects of such compounds (e.g., Gould, 1979; Lindroth, in prep.), but the mechanisms involved are unknown.

Since differences between species in their tolerance to such compounds is at least partially understood, inferences can be drawn about the causes of variation at the intraspecific level. In the case of tiger swallowtail larvae that can feed on umbellifers containing furanocoumarins, mixed function oxidases are partially responsible for the caterpillar's tolerance to these potent insecticidal compounds (Ivie et al., 1983). The tobacco hornworm that can tolerate high levels of nicotine is less neurologically sensitive to the compound and excretes it rapidly (Self et al., 1964). The biochemical genetic literature on insecticide resistance indicates that changes in levels of mixed function oxidases (as well as qualitative changes in isoenzyme forms) can be controlled by single genes (Plapp, 1976). Similarly, it has been shown with dipterans that decreased sensitivity of nerve tissues to insecticides can be controlled by single genes. By combining all types of information, it is possible to conclude that single gene transfers could unintentionally affect the host range of an herbivore. The probability of such an effect could be great or infinitely small, depending on the product coded for by the gene.

B. Animals

1) Microbes

Microbial interactions with animals may involve pathogenesis, symbiosis, or a third type of interaction called amensalism where the animal is unaffected by the microbe, but serves to nourish the microbe or to transfer the microbe to other animal or plant hosts. The three-way

interaction of animal #1, microbe, animal #2 (or plant) is quite common and will be addressed after the simpler, two-species interactions are considered.

For obvious reasons, microbial host interactions which either positively or negatively affect human health have received the most detailed scientific scrutiny. Thus, the volume and sophistication of the literature on genetics of pathogenicity of a few bacterial genera (i.e., Escherichia, Neisseria, Staphylococcus) far surpasses that on all other microbes. It is instructive to look at some of the details of E. coli pathogenicity as a means of gaining insight into the type of factors that may govern other microbe/host systems.

Most E. coli strains are innocuous inhabitants of vertebrate digestive tracts. However, there are some strains that are associated with meningitis and general infections in humans (e.g., Cheasty et al., 1977), other mammals (e.g., Glode et al., 1977), and birds (e.g., Smith and Huggins, 1980). The capacity of these strains to survive and reproduce in vertebrate tissues and body fluids requires an ability to overcome host defenses and obtain nutrients. Many studies were conducted in the 1970's to determine what characteristics differentiated E. coli strains that were capable of causing infections from those that were benign (e.g., Robbins et al., 1974; Smith, 1974; McCabe et al., 1978; Williams, 1979). From this work it became obvious that multiple factors could contribute to pathogenicity.

One particular capsular (K) polysaccharide antigen, K1, was found in 80% of the E. coli isolates from neonatal meningitis (Robbins et al., 1974). A number of lipopolysaccharides with repeating oligosaccharide side chains that gave them their specific serological properties were also implicated in pathogenicity (Orskov et al., 1977). The role of these two types of antigens was thought to be in generally shielding the bacteria from antibodies and a goup of about 20 serum proteins (i.e., complement). Yet, the fact that only a small fraction of the approximately 400 known K antigens were associated with virulence indicated that small genetic changes that code for production of these antigens may be very important in determining pathogenicity (Ogata, 1983). While these antigens were coded for by bacterial DNA, another factor associated with pathogenicity (traT membrane protein) was coded for by a plasmid.

Moving from correlational data to actual cause and effect involved detailed work in which bacterial strains were synthesized that contained different combinations of pathogenicity associated factors. Smith and Huggins (1980), who pioneered this approach, found that a pathogenic strain with the K antigen removed was far less pathogenic than the parent strain. Removal of the O antigen or the plasmid also decreased pathogenicity. However, when all three of these factors were added to a non-pathogenic strain, the increase in pathogenicity was very small. These results indicate that not all the factors contributing to

pathogenicity have been discovered. They also indicate that genes for pathogenicity interact epistatically. This knowledge is very important with regard to genetic engineering, because it may be possible to "disarm" a bacterial strain by removing a number of virulence factors. For the disarmed bacteria to regain a significant amount of virulence would require the acquisition of multiple traits. Smith and Huggins (1978) used their results as supportive evidence of the safety of using their nonpathogenic (K-12) strain in recombinant DNA work.

Although the finding of multiple, epistatically interacting virulence factors can be viewed as a safety feature of the E. coli system, there are at least four caveats to keep in mind. First, it has been found that the effect of a single virulence factor in increasing pathogenicity is strain dependent (e.g., Taylor and Robinson, 1980). Second, more recent work by Smith and Huggins (1985) indicates that for some hosts (chickens, ducks, guinea pigs) the K-12 strain can be made highly virulent by adding the O,K and plasmid-borne factor. Thus, not only are the effects of virulence factors strain-dependent, they are host-dependent as well. Third, single plasmids have been found that contain genes coding for more than one virulence factor, so rapid acquisition of a number of virulence factors is possible. For example, the ColV plasmid can code for the membrane protein, traT, as well as a novel iron uptake system that significantly increases pathogenicity

(Williams, 1979). Fourth, since the effects of virulence factors may be almost totally epistatic, it is difficult to determine whether a given avirulent bacterial strain lacks 1 or 20 virulence factors.

While the work on E. coli is more complete than that on the pathogenicity of other microbes, it is interesting to note some of the similarities that have been found between virulence factors in E. coli and those in other microbes. For example, Neisseria meningitidis produces a polysaccharide that is correlated with virulence (Masson et al., 1982). An iron chelating enterochelin found in E. coli is also associated with virulence of Salmonella typhimurium (Yancey et al., 1979). Similarly, a plasmid that codes for an iron-binding system is associated with virulence of the fish pathogen, Vibrio anguillarum (Crosa, 1980). In contrast to the cases above in which virulence depended primarily on genes coding for escaping host defenses and sequestration of a rare nutrient, the virulence of Staphlococcus aureus has been linked to the production of a specific protein, exotoxin, that debilitates the host (Ritz et al., 1984).

Given the somewhat complicated genetic and physiological changes associated with microbial pathogenicity, the recurring question of ecological and physiological costs must be addressed. What is the likelihood that strains carrying genes for pathogenicity persist in the normal habitats of the species (e.g., the digestive track)?

As with genetic traits influencing the host range of organisms in other phyla and environments, rigorous studies are rare and the answer seems to be case dependent. In one study by Williams (1979), E. coli strains with plasmid coded, iron-sequestering ability reproduced much faster than those lacking this trait when placed in an environment containing a low concentration of iron. When placed in an environment containing excess iron (where the sequestering ability was presumably unnecessary), reproduction was equal in the two strains, indicating a lack of cost involved in harboring this trait. Of course this information begs the question of why don't most E. coli strains harbor the plasmid coding for this trait if it only confers advantages and no disadvantages?

If the literature on genetics of microbes associated with humans is generalizable, the fact that a given microbial species is usually nonpathogenic does not rule out the possibility that it could be transformed to a pathogenic condition. Such pathogenic forms would at least in some cases be capable of competing with nonpathogenic strains.

In addition to genetics of the microbial virulence, it is important to understand the genetics of host suitability (or susceptibility). A number of genetic studies of mammalian susceptibility to bacterial infection indicate that as in the microbes themselves, a number of interacting genes are involved (Skamene, 1983). Single genes have been found to be associated with a change from host susceptibility to

resistance in at least five bacterial genera (Hirst and Wallace, 1976; Cheers and McKenzie, 1978; Groves and Osterman, 1978; Plant, 1979). In some cases the chromosomal locations of these genes have actually been mapped (Skamene, 1983). These genes have been found to regulate both immune and preimmune phases of resistance (e.g., Nacy and Groves, 1981; Pelletier et al., 1982), but the interactions of these genes have not been studied in detail.

The above examples dealt with microbial/host interactions that required only one type of host species. As an example of a microbial/host system involving more than one host, the case of arboviruses is useful. Arboviruses are by definition arthropod-borne viruses. The arthropods that move these viruses between other plant or animal hosts are not significantly debilitated by the virus, although the virus does multiply within it. The genetics of mosquito strain suitability as vectors has been studied from a number of perspectives and can serve as a model for this type of genetic interaction.

Genetic variation has been found in the competence of mosquito species to transmit alpha-, flavi-, and bunyviruses as well as malaria (Hardy et al., 1983). The physiological correlates of these genetic differences have been worked out in some cases and indicate that a barrier between the gut and the thoracic cavity is often involved (e.g., Mariani, 1961; Gubler et al., 1979). In other cases enzymatic

degradation in the gut, or a barrier to salivary gland infection that is necessary for transmission have been implicated (Houk and Hardy, 1981; Hardy et al., 1983).

In some cases genetically controlled barriers appear to be specific to certain viruses (Hardy et al., 1983). In one case, however, a single genetic factor seems to control a barrier to four different viruses (Gubler and Rosen, 1976). The number of genes controlling virus vector competence has not been well established, but single genes may exert strong effects (Hardy et al., 1983).

Studies of geographic variation in vector competence indicate that there may be differentiation among geographically proximate strains. In one detailed study the variation for oral infection within a single strain of Aedes aegypti for yellow fever virus was almost equal to the variation in samples collected from around the world (Tabachnik et al., 1985; Wallis et al., 1985).

The genetics of malaria vector competence is somewhat similar. An important recent study found that it was possible to select a single strain of Anopheles gambia mosquito for lack of ability to transmit malaria (Collins et al., 1986). In this study, plasmodium oocytes were encapsulated by the non-transmitting genotypes. Simple genetic control mechanisms were implicated, although not proven to be controlling variation in the encapsulating ability of the strain. Although the non-

transmitting strain was a poor vector of a number of species of plasmodium, there appeared to be strong genetic variation within some species of plasmodium regarding their ability to avoid encapsulation.

Arthropods also serve as vectors for plant viruses of importance to agriculture. Although few studies have investigated the genetic variation in vector competence of these arthropods, it is interesting to note that one of the earliest studies on genetics of vector competence was conducted on a leafhopper that transmitted maize-streak virus (Story, 1932).

Parasitic animals

Parasitic higher invertebrates are quite common, but the literature on genetic variability in these organisms appears to be scant. In some of the earlier evolutionary ecology work it was thought that head lice and body lice of humans were genetic variants of a single species, but it was later found that they were indeed two species.

Some work has been done on strains of the insect parasitoid wasps of the genus Trichogramma (Thompson, 1986 and references within). There seems to be some differentiation of these wasps in terms of host species preference, but detailed genetic experiments have not been conducted.

CONCLUSIONS

Given all the information reviewed in this document, what general statements can be made about potential risks involved in the environmental release of genetically engineered organisms?

1. There are a large number of habitat and host range changes, mediated by the effects of single genes. These single gene effects on habitat and host range are found in organisms as diverse as bacteria, plants and animals. Thus, it is possible that the transfer of a single gene into a species (or isolated populations) could change the abundance and distribution of the recipient species.

2. Since both addition and deletion of single genes can influence host range, and many genes can have unanticipated pleiotropic effects, absolute statements about lack of risk associated with certain general types of genetic modifications are presently unreasonable.

3. It may be possible to rank the risk involved in specific cases based on assessment of the known ecological properties coded for by a given gene, as long as it is realized that the side effects of a gene in one species or population may differ from those in another species or population.

4. Since species' barriers to gene flow are not absolute, it is feasible that a trait engineered into one species could, after environmental release, be transferred to other species and affect their ecology.

5. Disarming of recipient microbes by adding conditionally lethal single gene traits could be useful in limiting risks, especially if a number of conditional lethals were added and the microbes were to be used in a highly restricted environment, such as a fermenting tank.

6. Since a single plasmid can carry a number of genes coding for different types of ecological traits (e.g., iron sequestration and other proteins), care must be exercised in characterizing genes carried by the plasmid other than the gene of direct interest.

7. The transfer of genes between organisms in different phyla is more likely to introduce novel traits than transfer of genes between conspecifics from different populations. However, a gene moved from the genetic environment of one population to the genetic environment of another population may have novel effects on host range and habitat related traits. Furthermore, since many of the organisms to be involved in genetic engineering are asexual, each clone or population may be viewed as an independently evolving species. This fact tends to diminish the utility of the species concept in establishing policy guidelines.

References

Abraham, E. P., E. Chain, C. M. Fletcher, H. W. Florey, A. D. Gardner, N. G. Heatley, and M. A. Jennings. 1941. Further observations on penicillin. Lancet ii: 177-189.

Akiba, T., K. Koyama, Y. Kimura, and T. Fukushima. 1960. On the mechanism of development of multiple drug-resistant clones of Shigella. Jpn. J. Microbiol. 4: 219-227.

Alexander, H. M. 1987. Spatial heterogeneity and disease in natural populations. In Plant Epidemiology. (In press)

Antonovics, J. 1984. In Perspectives in Plant Population Ecology, Dirzo, R. and J. Sarukhan [eds.]. Sinauer Publ., Sunderland, Mass. pp. 213-228.

Bradshaw, A. D. 1984. In Perspectives in Plant Population Ecology, Dirzo, R. and J. Sarukhan [eds.]. Sinauer Publ., Sunderland, Mass. pp. 213-228.

Bradshaw, A. D. and McNeilly, T. 1981. Evolution and Pollution. Arnold Publ., London.

Breese, E. Z. and Charles, A. H. 1962. Population studies in rye grass. Rep. Welsh Pl. Breed. Stn., pp. 30-34.

Briggs, D. and Walters, S. M. 1984. Plant Variation and Evolution. Cambridge Univ. Press, Cambridge.

Brock, T. D. 1985. Life at high temperatures. Science 230: 132-138.

Brock, T. D. and M. L. Brock. 1966. Temperature optima for algal development in yellowstone and iceland hot springs. Nature 209: 733-734.

Brougham. R. W. and Harris, W. 1967. Rapidity and extent of change in genotypic structure induced by grazing in a rye grass population. N.Z. J. Agric. Res. 10: 56-65.

Carson, H. L. & A. T. Ohta. 1981. Origin of the genetic basis of colonizing ability, pp. 365-370. In G. G. E. Scudder & J. L. Reveal [eds.], Evolution Today. Carnegie Mellon Univ., Pittsburg.

Campbell, L. L., Jr. 1955. Purification and properties of an alpha-amylase from facultative thermophilic bacteria. Arch. Biochem. Biophys. 54: 154-161.

Chain, E., H. W. Florey, A. D. Gardner, N. G. Heatley, M. A. Jennings, J. Orr-Ewing, and A. G. Sanders. 1940. Penicillin as a chemotherapeutic agent. Lancet ii: 226-228.

Cheasty, T., R. J. Gross, and B. Rowe. 1977. Incidence of K1 antigen in Escherichia coli isolated from blood and cerebro-spinal fluid of patients in the United Kingdom. J. Clinic. Pathol. 30: 945-947.

Cheers, C. and I. F. C. McKenzie. 1978. Resistance and susceptibility of mice to bacterial infection: genetics of listeriosis. Infect. Immun. 19: 755-762.

Clausen, J., Keck, D. D., and Hiesey, W. M. 1948. Environmental response of climatic races of Achillea. Can. Inst. Wash. Publ. 581.

Clewell, D. B. 1981. Plasmids, drug resistance, and gene transfer in the genus Streptococcus. Microbiol. Rev. 45: 409-436.

Collins, F. H., R. K. Sakai, K. D. Vernick, S. Paskewitz, D. C. Seeley, L. H. Miller, W. E. Collins, C. C. Campbell, R. W. Gwadz. 1986. Genetic selection of a Plasmodium-refractory strain of the malaria vector Anopheles gambiae. Science 234: 607-610.

Cook, S. A., and Johnson, M. P. 1968. Adaptation to heterogenous environments. Evolution 22: 496-516.

Cormstock, V. E., and Anderson, R. N. 1968. An inheritance study of tolerance in flax treated with MCPA. Crop Science 8: 423-427.

Crane, M. B., and Lawrence, W. J. C. 1934. The Genetics of Garden Plants. Macmillan Publ., London.

Crosa, J. H. 1980. A plasmid associated with virulence in the marine fish pathogen Vibrio anguillarum specifies an iron-sequestering system. Nature 284: 566-568.

Crumpacker, D. W. 1967. Genetic load in maize (Zea mays) and other cross-fertilized plants and animals. Evol. Biol. 1: 306-424.

DaCosta, C. P. and C. M. Jones. 1971. Cucumber beetle resistance and mite susceptibility controlled by the bitter gene in Cucumis sativus L. Science 172: 1145-1146.

Daum, R. S., V. P. Syriopoulou, A. L. Smith, D. W. Scheifele, and J. E. Willard. 1981. Loss of plasmid DNA coding for thermal expansion coefficient of volume -lactamase during experimental infection with Haemophilus influenzae type b. J. Infectious Dis. 143: 548-553.

Davies, M. S., and Snaydon, R. W. 1976. Rapid population differentiation in a mosaic environment. Heredity 36: 59-66.

Denny and VanEtten 1982.

Dirzo, R. 1984. In Perspectives in Plant Population Ecology, Dirzo, R. and Sarukhan, J. [eds]. Sinauer Publ., Sunderland, Mass. pp. 213-228.

East, E. M. 1916. Studies on size inheritance in Nicotiana. Genetics 1: 164-176.

Ehrlich, P. 1959. Chemotherapy, pp. 505-518. In The Collected Papers of Paul Ehrlich, Vol. 3. Pergam, London.

Falconer, D. S. 1981. Introduction to Quantitative Genetics, Second Edition. Longman, New York. 340 pp.

Ferry, R. L. and G. G. Kennedy. 1987. Genetic analysis of 2-tridecanone concentration leaf trichome characteristics and tobacco hornworm resistance in Lycopersicon. J. Am. Soc. Hort. Sci. (In press)

Flor, H. H. 1942. Inheritance of pathogenicity in Melampsora lini. Phytopathology 32: 653-659.

Flor, H. H. 1971. Current status of the gene-for-gene concept. Ann. Rev. Phytopathol. 9: 275-295.

Futuyma, D. J. 1979. Evolutionary Biology. Sinauer Assoc., Sunderland, Massachusetts.

Gartside, P. W., and McNeilly, T. 1974. The potential for evolution of heavy metal tolerance in plants. Heredity 32: 335-348.

Georghiou, G. P. 1986. The magnitude of the resistance problem, pp. 14-43. In E. H. Glass [Chairman], Pesticide Resistance. Strategies and Tactics for Management. National Academy Press, Washington, D.C.

Glode, M. P., A. Sutton, E. R. Moxon, and J. B. Robbins. 1977. Pathogenesis of neonatal Escherichia coli meningitis: induction of bacteremia and meningitis in infact rats fed E. coli K1. Infect. Immun. 16: 75-80.

Gould, F. 1979. Rapid host range evolution in a population of the phytophagous mite, Tetranychus urticae Koch. Evolution 33: 791-802.

Gould, F. 1983. Genetics of plant-herbivore systems: interactions between applied and basic study, pp. 599-653. In R. Denno and B. McClure, Variable Plants and Herbivores in Natural and Managed Systems. Academic Press, New York.

Gould, F. 1984. Role of behavior on the evolution of insect adaptation to insecticides and resistant host plants. Bull. Ent. Soc. Am. 30: 34-41.

Groves, M. G. and J. V. Osterman. 1978. Host defenses in experimental scrub typhus: genetics of natural resistance to infection. Infect. Immun. 19: 583-588.

Gubler, D. J. and L. Rosen. 1976. Variation among geographic strains of Aedes albopictus in susceptibility to infection with dengue viruses. Am. J. Trop. Med. Hyg. 25: 318-325.

Gubler, D. J. and L. Rosen. 1976. A simple technique for demonstrating transmission of dengue virus by mosquitoes without the use of vertebrate hosts. Am. J. Trop. Med. Hyg. 25: 146-150.

Gubler, D. J., S. Nalim, R. Tan, H. Saipan, and J. S. Saroso. 1979. Variation in susceptibility to oral infection with dengue viruses among geographic strains of Aedes aegypti. Am. J. Trop. Med. Hyg. 28: 1045-1052.

Hamrick, J. L. 1979. In Topics in Plant Population Biology, Solmig, O. T., Jain, S., Johnson, G. and Raven, P. [eds.]. Columbia Univ. Press, New York. pp. 84-113.

Hardy, J. L., E. J. Houk, L. D. Kramer, and W. C. Reeves. 1983. Intrinsic factors affecting vector competence of mosquitoes for arboviruses. Annu. Rev. Entomol. 28: 229-262.

Hardy, R. W. F. 1986. Biotechnology in pesticide resistance development, p. 130-141. In E. H. Glass [Chairman], Pesticide Resistance. Strategies and Tactics for Management. National Academy Press, Washington, D.C.

Harlan, H. V., and Martini, M. L. 1938. The effect of natural selection on a mix of barley varieties. J. Agric. Res. 57: 189-199.

Hayes, W. 1968. The Genetics of Bacteria and Their Viruses. John Wiley & Sons Inc., New York.

Hedges, R. W. and A. E. Jacob. 1974. Transposition of ampicillin resistance from RP4 to other replicons. Molecular Gen. Genet. 132: 31-40.

Hickey, D. A., and McNeilly, T. 1975. Competition between metal tolerant and normal plant populations. A field experiment on normal soil. Evolution 29: 458-464.

Hirst, R. G. and M. E. Wallace. 1976. Inherited resistance to Corynebacterium kutscheri in mice. Infect. Immun. 14: 475-482.

Horsfall, J. G. and E. B. Cowling. 1979. Plant Disease. An Advanced Treatise, Vol. IV. Academic Press, New York. 466 pp.

Horsfall, J. G. and E. B. Cowling. 1980. Plant Disease. An Advanced Treatise, Vol. V. Academic Press, New York. 534 pp.

Houck, E. J. and J. L. Hardy. 1981. p-nitrophenylacetate hydrolysis by the nonspecific esterases of the mosquito, Culex tarsalis Coquillett. Insect Biochem. 11: 97-102.

Huettel, M. D. 1987. Evolutionary genetics of arthropod behavior. Plenum Press, New York. (In press)

Ivie, G. W., D. Bull, R. C. Beier, N. W. Pryor, and E. H. Oertli. 1983. Metabolic detoxification: mechanism of insect resistance to plant psoralens. Science 221: 374-376.

Jennings, P. R. and de Jesus, J. 1968. Studies on competition in rice. Evolution 22: 119-124.

Jones, D. A. 1966. On the polymorphism of cyanogenesis in Lotus corniculatus. Selection by animals. Can. J. Genet. Cytol. 8: 556-567.

Jones, D. A. 1968. On the polymorphism of cyanogenesis in Lotus corniculatus L. II. The interaction with Trifolium repens L. Heredity 23: 453-455.

Jones, D. A. 1972. On the polymorphism of cyanogenesis in Lotus corniculatus L. IV. The Netherlands. Genetica 43: 394-406.

Jones, D. A. 1973. On the polymorphism of cyanogenesis in Lotus corniculatus L. V. Denmark. Heredity 30: 381-386.

Jones, L. S., F. N. Briggs & R. A. Blanchard. 1950. Inheritance of resistance to the pea aphid in alfalfa hybrids. Hilgardia 20:

Keiding, J. 1986. Prediction or resistance risk assessment, p. 279-297. In E. H. Glass [Chairman], Pesticide Resistance Strategies and Tactics for Management. National Academy Press, Washington, D.C.

von Kerner, M. 1891. Planzenleben, Vol. 2. Leipzig, E. Germany.

Kettlewell, B. 1973. The Evolution of Melanism. Clarendon Press, Oxford.

Koch, A. L. 1981. Evolution of antibiotic resistance gene function. Microbiol. Rev. 45: 355-378.

Kondo, I., H. Nakahara, and T. Ishikawa. 1975. The possibility of a specific plasmid mediating metal resistances, pp. 145-152. In S. Mitsuhashi and H. Hashimoto [eds.], Microbial Drug Resistance. University Park Press, Baltimore.

Lamberti, F., J. M. Waller, and N. A. Van der Graaff. 1981. Durable Resistance in Crops. Plenum Press, New York.

Laster, M. L., D. F. Martin, and D. W. Parvin, Jr. 1976. Potential for suppressing tobacco budworm (Lepidoptera: Noctuidae) by genetic sterilization. Miss. Agric. Exp. Stn. Tech. Bull. 82: 1-9.

Laster, M. L., S. D. Pair, and D. F. Martin. 1982. Acceptance and development of Heliothis subflexa and H. virescens (Lepidoptera: Noctuidae), and their hybrid and backcross progeny on several plant species. Environ. Entomol. 11: 979-980.

LeBaron, H. M., and Gressel, J. 1982. Herbicide Resistance in Plants. John Wiley, New York.

Leonard, K. J. 1986. The host population as a selective factor, pp. 163-179. In M. S. Wolfe and C. E. Caten [eds.], Populations of Plant Pathogens: Their Dynamics and Genetics. Blackwell, Oxford.

Levin, D. A. 1978. The origin of isolating mechanisms in flower plants. Evol. Biol. 11: 185-317.

Levin, D. A. 1984. In Perspectives in Plant Population Ecology, Dirzo, R. and Sarukhan, J. [eds.]. Sinauer Publ., Sunderland, Mass. pp. 213-228.

Linhart, Y. B. 1974. Intra-population differences in annual plants. Evolution 28: 232-243.

Lukefahr, M. J. & D. F. Martin. 1966. Cotton plant pigments as a source of resistance to the bollworm and tobacco budworm. J. Econ. Entomol. 59: 176-179.

Mariani, M. 1961. Membrana peritrofica e enfettabilitia deglianofeli. Riv. Malariol. 40: 243-246.

Masson, L., B. E. Holbein, and F. E. Ashton. 1982. Virulence linked to polysaccharide production in serogroup B Neisseria meningitidis. FEMS Microbiol. Letters 13: 187-190.

Matthews, D. E. and H. D. VanEtten. 1983. Detoxification of the phytoalexin pisatin by a fungal cytochrome P-450. Archives Biochem. Biophys. 224: 494-505.

McCabe, W. R., B. Kaijser, S. Obling, M. Uwaydah, and L. A. Hanson. 1978. Escherichia coli in bacteremia: K and O antigens and serum sensitivity of strains from adults and neonates. J. Infect. Dis. 138: 33-41.

McKenzie, J. A., M. J. Whitten and M. A. Adena. 1982. The effect of genetic background on the fitness of diazinon resistance genotypes of the Australian sheep blowfly, Lucilia cuprina. Heredity 49: 1-9.

Mitsuhashi, S. and H. Hashimoto. 1975. Microbial Drug Resistance. University Park Press, Baltimore.

Morrison, D. A. and Myerscough, P. J. 1982. Genecological differentiation, in Leptospermum flavescens. Aust. J. Biol. Sci. 11: 761-764.

Nacy, C. A. and M. G. Groves. 1981. Macrophages in resistance to rickettsial infections: early host defense mechanisms in experimental scrub typhis. Infect. Immun. 31: 1239-1350.

Nelson, R. R. 1979. The evolution of parasitic fitness. In J. G. Horsfall and E. B. Cowling [eds.], Plant Disease, Vol. IV. Academic Press, New York.

Nilius, A. M. and D. C. Savage. 1984. Serum resistance encoded by colicin V plasmids in Escherichia coli and its relationship to the plasmid transfer system. Infect. Immun. 43: 947-953.

Nilsson-Ehle, H. 1909. Kreazungsun tersuchungen on Hafer und Weizen. Lunds, Univ. Arsskr. 2: 1-122.

Ochiai, K., T. Yamanaka, K. Kimura, and O. Sawada. 1959. Inheritance of drug resistance (and its transfer) between Shigella and E. coli strains. Nihon Iji Shimpo 1861: 34. (In Japanese)

Ogata, R. T. 1983. Factors determining bacterial pathogenicity. Clin. Physiol. Biochem. 1: 145-159.

Orskov, I., F. Orskov, B. Jann, and K. Jann. 1977. Serology, chemistry, and genetics of O and K antigens of Escherichia coli. Bacteriol. Rev. 41: 667-710.

Ouchi, S. 1983. Induction of resistance or susceptibility. Annu. Rev. Phytopathol. 21: 289-315.

Parlevliet, J. E. 1983. Models explaining the specificity and durability of host resistance derived from the observations on the barley-Puccinia hordei system, pp. 57-80. In F. Lamberti, J. M. Waller & N. A. Van der Graaf, Durable Resistance in Crops. Plenum, New York.

Pelletier, M., A. Forget, D. Bourassa, P. Gros, and E. Skamene. 1982. Immunopathology of BCG infection in genetically resistant and susceptible mouse strains. J. Immun. 129: 2179-2185.

Plant, J. and A. A. Glynn. 1976. Genetics of resistance to infection with Salmonella typhinurium in mice. J. Infect. Dis. 133: 72-78.

Plant, J. and A. A. Glynn. 1979. Locating Salmonella resistance gene in mouse chromosome 1. Clin. Exp. Immunol. 37: 1-6.

Plapp, F. W. 1976. Biochemical genetics of insecticide resistance. Annu. Rev. Entomol. 21: 179-197.

Plapp, F. W. 1986. Genetics and biochemistry of insecticide resistance in arthropods: prospects for the future, pp. 73-86. In E. H. Glass [Chairman], Pesticide Resistance. Strategies and Tactics for Management. National Academy Press, Washington, D.C.

Quesnel, L. B. and A. D. Russell [eds]. 1983. Introduction, pp. 1-18. In Antibiotics: Assessment of Antimicrobial Activity and Resistance. Academic Press, New York.

Rathcke, B. J. and R. W. Poole. 1975. Coevolutionary race continues: butterfly larval adaptation to plant trichomes. Science 187: 175-176.

Reynard, A. M., M. E. Beck, and R. K. Cunningham. 1978. Effects of antibiotic resistance plasmids on the bactericidal activity of normal rabbit serum. Infect. Immun. 19: 861-866.

Richmond, M. H. 1977. The survival of R-plasmids in the absence of antibiotic selection pressure, pp. 61-77. In J. Drews and G. Hozenauer [eds.], R-Factors: Their Properties and Possible Control. Springer-Verlag, New York.

Ritz, H. L., J. J. Kirkland, G. G. Bond, E. K. Warner, and G. P. Petty. 1984. Association of high levels of serum antibody to staphylococcal toxic shock antigen with nasal carriage of toxic shock antigen-producing strains of Staphylococcus aureus. Infect. Immun. 43: 954-958.

Robbins, J. B., G. H. McCracken, Jr., E. C. Gotschlich, F. Orskov, I. Orskov, and L. A. Hanson. 1974. Escherichia coli K1 capsular polysaccharide associated with neonatal meningitis. N. Engl. J. Med. 290: 1216-1220.

Roush, R. T. and B. A. Croft. 1986. Experimental population genetics and ecological studies of pesticide resistance in insects and mites, pp. 257-270. In E. H. Glass [Chairman], Pesticide Resistance. Strategies and Tactics for Management. National Academy Press, Washington, D.C.

Saunders, G. F. and L. L. Campbell. 1966. Characterization of a thermophilic phage for Bacillus stearothermophilus. J. Bact. 91: 340-348.

Schooler, A. B., Bell, A. R., and Natewaja, J. D. 1972. Inheritance of siduron tolerance in foxtail barley. Weed Sci. 20: 167-172.

Scott, K. R. and Putwain, P. D. 1981. Maternal inheritance of simazine resistance in a population of Senecio vulgara. Weed Res. 21: 137-143.

Self, L. S., F. E. Guthrie, and F. Hodgson. 1964. Metabolism of nicotine by tobacco-feeding insects. Nature 204: 300-301.

Sequeira, L. 1980. Defenses triggered by the invader: recognition and compatibility phenomena, pp. 179-200. In J. G. Horsfall and E. B. Cowling [eds.], Plant Disease. An Advanced Treatise, Vol. V. Academic Press, New York.

Showers, W. B., G. L. Reed, and H. Oloumi-Sadeghi. 1974. European corn borer: attraction of males to synthetic lure and to females of different strains. Environ. Entomol. 3: 51-58.

Sinnott, E. W., and Hammond, D. 1930. Factorial balance in the determination of fruit shape in Cucurbita. Amer. Nat. 64: 509-524.

Skamene, E. 1983. Genetic regulation of host resistance to bacterial infection. Rev. Infect. Dis. (Suppl.) 5: S823-S832.

Skinner, F. A. 1968. The limits of microbial existence, pp. 6-18. In D. W. Thayer [ed.], Microbial Interaction With the Physical Environment. Halstead Press, Stroudsburg, PA.

Smith, H. W. 1974. A search for transmissible pathogenic characteris in invasive strains of Escherichia coli: the discovery of a plasmid-controlled toxin and a plasmid-controlled lethal character closely associated, or identical, with colicin V. J. Gen. Microbiol. 83: 95-111.

Smith, H. W. and M. B. Huggins. 1978. The influence of plasmid-determined and other characteristics of enteropathogenic Escherichia coli on their ability to proliferate in the alimentary tracts of piglets, calves and lambs. J. Med. Microbiol. 11: 471-492.

Smith, H. W. and M. B. Huggins. 1980. The association of the O18, K1 and H7 antigens and the ColV plasmid of a strain of Escherichia coli with its virulence and immunogenicity. J. Gen. Microbiol. 121: 387-400.

Smith, H. W. and M. B. Huggins. 1985. Acquisition of genes from an O18:K1:H7 ColV+ strain of Escherichia coli renders intracranially-inoculated E. coli K12 highly virulent for chickens, ducks and guinea-pigs but not mice. J. Hyg., Camb. 95: 363-374.

Smith, R. A. H., and Bradshaw, A. D. 1979. The use of metal tolerant plant populations for the reclamation of metalliferous wastes. J. Appl. Ecol. 16: 595-612.

Snaydon, R. W. 1962. The growth and competitive ability of contrasting natural populations of Trifolium repens. J. Ecol. 50: 439-447.

Snaydon, R. W. 1970. Rapid population differentiation in a mosaic environment. Evolution 24: 257-269.

Snaydon, R. W., and Bradshaw, A. D. 1961. Differential response to calcium with the species Festuca ovina. New Phytolog. 60: 219-234.

Snaydon, R. W., and Davies, M. S. 1976. Rapid population differentiation in a mosaic environment. Heredity 37: 9-25.

Snaydon, R. W., and Davies, M. S. 1982. Rapid divergence of plant populations in response to recent changes in soil conditions. Evolution 36: 289-297.

Spencer, H. C. 1985. Drug-resistant malaria-changing patterns mean difficult decisions. Trans. Roy. Soc. Trop. Med. Hyg. 79: 748-758.

Stebbins, G. L. 1971. Chromosomal Evolution in Higher Plants. Arnold, London.

Story, H. H. 1932. The inheritance by an insect vector of the ability to transmit a plant virus. Proc. R. Soc. London Ser. B 112: 46-60.

Tabachnick, W. J., G. P. Wallis, T. H. G. Aitken, B. R. Miller, G. D. Amato, L. Lorenz, J. R. Powell, and B. J. Beaty. 1985. Oral infection of Aedes aegypti with yellow fever virus: geographic variation and genetic considerations. Am. J. Trop. Med. Hyg. 34: 1219-1224.

Tauber, M. J., C. A. Tauber, and S. Masaki. 1984. Adaptations to hazardous seasonal conditions: dormancy, migration, and polyphenism, p. 149-183. In C. B. Huffaker and R. L. Rabb [eds.], Ecological Entomology. John Wiley, New York.

Taylor, P. W. and M. K. Robinson. 1980. Determinants that increase the serum resistance of Escherichia coli. Infect. Immun. 29: 278-280.

Tegtmeier, K. J. and H. D. van Etten. 1982. Phytopathology 72: 608-612.

Teramura, A. H., and Strain, B. R. 1979. Localized populational differentiation in photosynthetic response to temperature and irradiance in Plantago lanceolata. Can. J. Bot. 57:L 2559-2563.

Thayer, D. W. 1968. Introduction: the limits of microbial existence, pp. 1-5. In D. W. Thayer [ed.], Microbial Interaction With the Physical Environment. Halsted Press, Stroudsburg, PA.

Thompson, J. N. 1986. Patterns in coevolution, pp. 119-143. In A. R. Stone and D. L. Hawksworth [eds.], Coevolution and Systematics. Clarendon Press, Oxford.

Turkington, R. and Aarssen, L. W. 1984. In Perspectives in Plant Population Ecology. Dirzo, R., and Sarukhan, J. [eds.]. Sinauer Publ., Sunderland, Mass.

Vournakis, J. N. 1984. Application of recombinant DNA technology to the domestication of marine plants, pp. 81-103. In R. R. Colwell, A. J. Sinskey, and E. R. Pariser [eds.], Biotechnology in the Marine Sciences. Proc. First Annu. MIT Sea Grant Lecture and Seminar. Wiley & Sons, New York.

Wallis, G. P., T. H. G. Aitken, B. J. Beaty, L. Lorenz, G. D. Amato, and W. J. Tabachnick. 1985. Selection for susceptibility and refractoriness of Aedes aegypti to oral infection with yellow fever virus. Am. J. Trop. Med. Hyg. 34: 1225-1231.

Warwick, S. I., and Black, L. 1980. Unparental inheritance of atropine resistance in Chenopodium album. Can. J. Plant Sci. 60: 751-756.

Wiebes, J. J. 1979. Coevolution of figs and their insect pollinators. Annu. Rev. Ecol. Syst. 10: 1-12.

Williams, P. H. 1979. Novel iron uptake system specified by colv plasmids: an important component in the virulence of invasive strains of Escherichia coli. Infect. Immun. 26: 925-932.

Yancy, R. J., S. A. L. Breeding, and C. E. Lankford. 1979. Enterochelin (enterobactin): virulence factor for Salmonella typhimurium. Infect. Immun. 24: 174-180.

Table I

Some Economically Important Plant Species in which Genetic Variation for Herbivore Resistance Exists[a]

Plant	*Herbivore*	*Genetic control of variation*	*General type of resistance*	*Reference*
Zea mays (corn)	European corn borer	Polygenic	Antibiosis (DIMBOA)	Scott and Guthrie (1967), Russell *et al.* (1975)
	Corn earworm	Polygenic	Antibiosis, nonpreference, tolerance	Widstrom and McMillian (1973)
	Corn-leaf aphid	Conflicting reports	Antibiosis, nonpreference (DIMBOA?)	Long *et al.* (1976), Pathak and Saxena (1976), Chang and Brewbaker (1975)
	Maize stem borers	?	Antibiosis	Kalode and Pant (1967), Mathur and Jain (1972)
	Fall armyworm	Polygenic	?	Widstrom *et al.* (1972)
Oryza sativa (rice)	Brown planthopper	2 dominant and 2 recessive genes with independent effects	Antibiosis, nonpreference	Lakshminarayana and Khush (1977), Song *et al.* (1972)
	Green leafhopper	4 dominant genes and 1 recessive gene with independent effects	Antibiosis + ?	Krishnaiah (1975)
	Rice stem borer	Monogenic in some crosses, polygenic in others	Antibiosis, nonpreference (mechanical)	Oliver and Gifford (1975), Lee *et al.* (1974)
	Rice gall midge	Conflicting reports	Antibiosis	Heinrichs and Pathak (1981)
Triticum spp. (wheat)	Hessian fly	7 dominant genes, 1 recessive gene, with independent effects	Antibiosis	Gallun *et al.* (1975)
	Greenbug	1 + recessive genes	?	Abdel-Malik *et al.* (1966)
	Cereal-leaf beetle	Polygenic	Nonpreference, antibiosis (pubescence)	Ringlund and Everson (1968), Gallun *et al.* (1975)

	Wheat stem sawfly	1 major gene and modifiers	Antibiosis (solid stem)	McKenzie (1965), McNeal *et al.* (1971)
Hordeum vulgare (barley)	Hessian fly	1 dominant gene at low temperature, 2 epistatically interacting genes at high temperature	Antibiosis?	Olembo *et al.* (1966)
	Cereal-leaf beetle	1 recessive gene	Nonpreference	Hahn (1968)
Sorghum bicolor (sorghum)	Corn-leaf aphid	1 dominant gene	Antibiosis	Cartier and Painter (1956), Gallun and Khush (1980)
	Greenbug	1 dominant gene	Antibiosis	Weibel *et al.* (1972)
	Shoot fly	?	?	Starks and Doggett (1970)
Medicago sativa (alfalfa)	Spotted alfalfa aphid	Polygenic	Nonpreference, antibiosis, tolerance	McMurtry and Stanford (1960) Harvey *et al.* (1960)
	Pea aphid	1 + dominant genes (?)	Antibiosis + ?	Jones *et al.* (1950), Ortman *et al.* (1960)
Gossypium spp. (cotton)	Boll weevil	1 gene for some traits	Nonpreference (red leaves, frego bract)	Isely (1928), Stephens and Lee (1961)
	Jassids	1 dominant gene, modifiers, and 2 epistatically interacting genes	Antibiosis, nonpreference (pubescene, thickness of cells)	Knight (1952), Muttuthamby *et al.* (1969), and Batra and Gupta (1970)
	Corn earworm, tobacco budworm	1–few genes, depending on trait	Nonpreference, antibiosis (gossypol, tannin, other secondary compounds, glabrous leaves)	Lukefahr and Martin (1966), Wilson and Lee (1971), Waiss *et al.* (1981)
Cucurbits	Cucumber beetles	1 recessive gene	Nonpreference	DaCosta and Jones (1971)
	Two-spotted spider mite	1 dominant gene, modifiers	Antibiosis or nonpreference	Gould, (1978), dePonti (1978)
	Melon aphid	1 dominant gene in some tests	Tolerance, antibiosis	Bohn *et al.* (1973)
	Squash bug	Polygenic	Nonpreference	Benepol and Hall (1967), Novero *et al.* (1962)

(Continued)

Table 1 (*Continued*)

Plant	*Herbivore*	*Genetic control of variation*	*General type of resistance*	*Reference*
Rubus idaeus (raspberry)	European raspberry aphid	9 dominant genes with independent effects	Nonpreference, antibiosis	Keep and Knight (1967), Keep *et al.* (1970)
	American raspberry aphid	1 dominant gene	Nonpreference, antibiosis	Kennedy *et al.* (1973), Daubeny (1966), Kennedy and Schaefers (1974)
Beta vulgaris (sugar beets)	Green peach aphid	Polygenic	Nonpreference, antibiosis	Russell (1978)
Lactuca sativa (lettuce)	Root aphid	?	Antibiosis	Dunn and Kempton (1974)
Malus robusta (apple)	Rosy apple aphid	1 dominant gene	Nonpreference (hypersensitivity)	Alston and Briggs (1970)
	Wooly apple aphid	1 dominant gene		Painter (1951)
	Rosy leaf-curling aphid	1 dominant gene	?	Alston and Briggs (1968)
Picea sp. (spruce)	Black-marked tussock moth	?	Avoidance	von Schonborn (1966)
	Spruce sawfly	?	Tolerance + ?	von Schonborn (1966)
	Spruce gall aphid	1 gene(?)	?	Thielges and Campbell (1972); Hanover (1980)
Pinus silvestris (Scots pine)	Shoot borer	?	?	Steiner (1974)
	Pine-root collar weevil	?	?	Wright and Wilson (1972)
	European pine sawfly	?	Antibiosis	Wright *et al.* (1976)

[a]The information presented represents probabilistic inferences that I, or the various authors referred to, have drawn concerning genetic control and mechanisms responsible for resistance. It is impossible in most of the cases presented to make absolute statements based on available data

Table 2.

Genetic Variation within Herbivore Species for Response to Host Plant Resistance[a]

Order and species of herbivore	*Host plant(s) involved in expression of variation*	*Mode of reproduction and genetic control of variation*	*Type of plant resistance involved*	*Scale of reported variation*	*Reference*
Hemiptera					
Amphorophora rubi (European raspberry aphid)	Raspberry	Seasonally parthenogenic, series of single dominant genes	NP + AB, series of single dominant genes	Within districts in England	Briggs (1965)
Schizaphis graminum (greenbug)	Wheat, barley	Parthenogenic, 4 biotypes	Single gene +	Within Texas	Wood (1971), Daniels (1981)
Rhopalosiphum maidis (corn-leaf aphid)	Sorghum, maize, wheat, barley, oats	Parthenogenic, 5 biotypes	NP + AB, monogenic + polygenic	Within Kansas	Wilde and Feese (1973), Cartier and Painter (1956), Pathak and Painter (1959)
Therioaphis maculata (spotted altalfa aphid)	Altalfa	Parthenogenic in some areas, 6 biotypes	NP, AB, I, polygenic	Within Canada, United States, and California	Stanford (1977), Nielson and Don (1974a,b)
Aphis gossypii (melon aphid)	Muskmelon	?	I + AB, single gene?	Populations from California, se United States	Ivanoff (1957), Kishaba *et al.* (1971)
Acyrthosiphon pisum (pea aphid)	Altalfa, pea	Parthenogenic in some areas (crosses performed, intricate results)	NP + AB, single gene +	Within United States, Canada, and local area of Germany	Cartier *et al.* (1965), Muller (1971), Muller and Hubert-Dahl (1973), Bournoville (1981)

(Continued)

Table 2 (continued)

Order and species of herbivore	*Host plant(s) involved in expression of variation*	*Mode of reproduction and genetic control of variation*	*Type of plant resistance involved*	*Scale of reported variation*	*Reference*
Aulacorthum solani (foxglove aphid)	Lungwort, foxglove, potato	Seasonally parthenogenic, additive on potato, dominance on foxglove and lungwort (in opposite directions)	AB + NP	Within local area of Germany	Muller (1976)
Urolecon caligatum	Goldenrod	Seasonally parthenogenic?	AB + ?	Within a field	Moran (1981)
Urolecon rudbeckiae	*Rudbeckia laciniata*	Seasonally parthenogenetic	AB + NP	Within local area in North Carolina	Service (1982)
Eriosoma langerum (wooly apple aphid)	Apple	Seasonally parthenogenic	Single gene	Within Australia	Sen Gupta and Miles (1975)
Nilaparvata lugens (brown planthopper)	Rice	Sexual polygenic? 3 biotypes?	AB + NP, series of 4 single genes	Philippines and se Asia	Claridge and den Hollander (1980), Lakshminarayana and Khush (1977), Sogawa, (1981a,b)
Nephotettix cincticeps (green rice leafhopper)	Rice	Sexual, 2 biotypes	NP + AB, series of 5 single genes	800 km	Sato and Sogawa (1981)
Nuculaspis californica (black pine leaf scale)	Ponderosa pine	Haplodiploid, additive?	AB	Local area	Alstad and Edmunds (Chapter 12)

Table 2 (continued)

Diptera					
Mayetiola destructor (Hessian fly)	Wheat	Sexual, series of single genes	AB, series of 8 single genes	A field, within United States	Painter *et al.* (1931), Gallun *et al.* (1975)
Pachydiplosis oryzae (rice gall midge)	Rice	Sexual, 4 biotypes	AB	India	Heinrichs and Pathak (1981)
Liriomyza sativae	Tomato, pea	Sexual	AB + NP	Within 1 county of North Carolina	S. Via (unpublished data)
Liriomyza brassicae	*Barbarea vulgaris, Thlaspi arvense, Brassica nigra*	Sexual	AB + NP	Within a field	Tavormina (1982; personal communication)
Coleoptera					
Cassidinae spp. (tortoise beetles)	Morning glory	Sexual	?	Within 1 county	M.D. Rausher (unpublished data)
Callosobruchus maculatus	Pigeon pea, adzuki bean	Sexual, additive	?	Lab population	Wasserman and Futuyma (1981)
Sitophilus oryzae	Split pea	Sexual, single recessive gene	AB	Worldwide	Coombs *et al.* (1977), Thind and Muggleton (1981)
Lepidoptera					
Colias philodice (clouded sulfur)	Alfalfa, vetch	Sexual	AB + ?	1 area of Colorado	Tabashnik (1982)
Laspeyresia pomonella (codling moth)	Apple, walnut, plum	Sexual	Avoidance in time	1 area of California	Phillips and Barnes (1975)
Alsophila pometaria (fall cankerworm)	Oaks, maple	Parthenogenetic	AB + NP	1 woodlot, New York, New Jersey	Mitter and Futuyma (Chapter 13)
Papilio polytenes (tiger swallowtail)	Carrot	Sexual	AB	United States, Costa Rica	Blau (1978)

(Continued)

Table 2 (continued)

Order and species of herbivore	*Host plant(s) involved in expression of variation*	*Mode of reproduction and genetic control of variation*	*Type of plant resistance involved*	*Scale of reported variation*	*Reference*
Papilio glaucus (tiger swallowtail)	Birch, aspen, tulip tree, cherry	Sexual, dominance	AB	United States, Canada	Scriber (Chapter 11)
Euphydryas editha (checkerspot)	*Pedicularis, Plantago, Collinsia*	Sexual	NP + AB	California	Singer (1970), Rausher (1982)
Acarina					
Tetranychus urticae (two-spotted spider mite)	Cucumber, potato, *Plantago*, tobacco	Haplodiploid, additive	AB, single gene + modifiers	Within 1 orchard and within United States	Gould (1978, 1979)

[a]Information on genetic control of variation in herbivore responses is even less precise than that for genetics of host resistance, so care must be taken in drawing absolute conclusions from this table. Biotypes of parthenogenic species may be equivalent to sibling species. AB, Antibiosis; NP, nonpreference; T, tolerance.

APPENDIX

Introduction

The first part of this document dealt with the issue of genetics of change in host range and habitat with a goal of estimating the possible risks to present ecosystem function related to the introduction of a genetically engineered organism. The following appended section deals with the potential of indigenous species of an ecosystem to genetically change in response to introduction of genetically engineered organisms. Since this section was written for a different audience and is intended for publication, it is more specific than the major part of this report and is added as what we consider an important appendix to this report.

It is our belief that while we must be concerned with containment of genetically engineered organisms, the potential genetic response of indigenous organisms to genetically engineered organisms may pose a problem of equal magnitude. This problem is worthy of far more attention than it has received to date.

This appendix offers a number of specific evolutionary approaches to the use of genetically engineered organisms. The general conclusion from this appendix is that if we are to avoid the rapid evolution of pests that are no longer suppressed by environmental release of bioengineered organisms, we must avoid widespread release of engineered crops or microbes that severely reduce fitness of pest populations (see Appendix for exceptions to this conclusion).

In terms of crop plants, it may be economically reasonable to put toxin coding genes in only 80% or less of the plants sown in a field. The use of mixtures of crop genotypes in a single field would not only inhibit evolution of adapted pests, it could also lead to reduced population size of microbial pest populations, and, importantly, it could increase yield by reducing intraspecific competition among the crop plants in a field. This last-mentioned effect would result from different plant genotypes engineered or classically bred to utilize slightly different niches in the soil profile or canopy. Differences among genotypes in terms of resistance to factors, such as drought stress, could lead to slightly reduced yields in years of high rainfall; but in years of drought, a portion of the crop would be resistant to yield loss. Over the long run, this would result in more stable yields.

Until recently, classical plant breeders have been aiming at production of genetically uniform cultivares. Although there are reasons for such a goal, classical plant breeders and genetic engineers must take into account the manifold benefits that could be accrued from breeding crops that are heterogenous for specifically targeted traits.

In addition to genetic heterogeneity among plants, phenotypic heterogeneity within plants regarding toxin production could be beneficial. Instead of using promotor sequences that lead to constituitive toxin production, it could be useful to find promotors that

would lead only to production of pest toxins in specific tissues, or at specific phenological stages of plant growth that corresponds with seasonal periods of damaging population levels of the pests.

Appendix #1

Fred Gould
Dept. of Entomology
840 Method Road
North Carolina State Univ
Raleigh, N. C. 27607

EVOLUTIONARY THEORY AND THE DESIGN OF GENETICALLY ENGINEERED CROPS

Fred Gould

Department of Entomology

North Carolina State University

Raleigh, North Carolina 27695

ABSTRACT

Recent transfer of genes conferring pest resistance to a crop plant demonstrates the potential of genetic engineering as a powerful tool in agriculture, but also raises questions regarding pest adaptation to engineered crops. Theoretical and empirical findings in evolutionary biology are drawn upon to evaluate a number of approaches for the design and deployment of genetically engineered, pest resistant crops. While some approaches are likely to impede pest adaptation and stabilize yields, others may cause problems reminiscent of the DDT era of pest control.

INTRODUCTION

Most of the controversy surrounding the use of genetically engineered organisms has focused on unintended establishment of these organisms, or their genes, in environments where they could disrupt ecological systems or adversely affect human health (eg. Gillette et al. 1985, Kolata 1985, Sharples 1982). In contrast, almost no attention has been devoted to potential, evolutionary responses of indigenous organisms to the genetically engineered organisms. These evolutionary changes could rapidly nullify the utility of the genetically engineered organisms and could significantly alter ecosystem function. My purpose here is to discuss how evolutionary theory could be used in developing release strategies that may limit such problems.

I will focus on the consequences of commercial use of genetically engineered crops containing genes that code for pest resistance traits. The two basic questions I will address are 1) Can we predict the evolutionary responses of pest organisms to genetically engineered crops? 2) Are there realistic tactics that can be used to minimize or beneficially manipulate the genetic responses of the pest organisms to genetically engineered crops? Although I will restrict this discussion to pest resistance genes, similar approaches could be taken whenever a genetically engineered organism affects the fitness of indigenous organisms.

A NEW TWIST TO AN OLD PROBLEM

In some ways there is nothing very new about incorporating genes for pest resistance into crop plants. Intentionally or unintentionally, humans have been doing this for hundreds of years by selectively collecting seed from plants in their fields that yielded best while faced with ablotic and biotic stresses (Harlan 1976). More

recently, agricultural scientists have been using classical quantitative genetic techniques to incorporate genes conferring pest resistance into modern cultivars (Russell 1978). These scientists have already been faced with the problem of pests nullifying the effectiveness of resistance genes by genetically adapting to them.

One important difference between past efforts to breed resistance factors into crops and the potential of genetic engineering techniques is in the gene pool available for tapping. Classical techniques for plant breeding transfer genetically controlled traits from one plant lineage to another during sexual reproduction. Although it is sometimes possible to cross plants in different species, there are, in general, very narrow taxonomic restrictions on the sources of genes that can be transferred via sexual gametes. If recent successes in genetic engineering are an indication of future prospects, it will be possible, not only to transfer genes for pest resistance between plants of different genera and families, but also between plants and organisms in different kingdoms (Comai and Stalker 1984). The recent transfer of a toxin produced by a bacteria (Bacillus thuringiensis) to tobacco plants underscores the ability of genetic engineers to cross taxonomic barriers and tap previously unavailable gene pools (Yanchinski 1986). Although to date, genetic engineers have concentrated on the transfer of genes coding for the production of toxic proteins, future transfers may include genes involved in synthesis of other types of toxins (Hardy 1986).

A second difference between classical and genetic engineering techniques lies in the potential number of resistance factors a crop may contain. Classical plant breeders are excited when they find and successfully transfer a single resistance factor to a modern cultivar. In the future, genetic engineers may be capable of producing

crops with a variety of new resistance factors (Comai and Stalker 1984). Since different plant families are noted for producing chemically distinct toxins and repellents (Rosenthal and Janzen 1979), genetically engineered crops could present pests with several novel, and therefore very potent, resistance factors.

Although I will discuss how these and other qualitative and quantitative differences between current and future breeding techniques may affect evolutionary responses of pests, there is much to be learned from past agricultural experiences in which pests have evolutionarily circumvented the effects of resistant crops.

NATURAL INCORPORATION OF RESISTANCE GENES

Another useful source of information regarding the potential of pests to adapt to genetically engineered crops comes from the natural occurance of plant resistance to insect and microbial herbivores (ie. pathogens). Incorporation of resistance has not been limited to human activities. An enormous number of biologically active chemicals are thought to be produced by natural plant populations as a result of evolutionary selection pressures to ward off "natural pest" damage. These pests have had millions of years to respond genetically, with counteradaptations to these plant defenses in a process that has broadly been referred to as a coevolutionary arms race (Gilbert 1971, Rathke and Poole 1975)

.

Theoretical and empirical study of these evolutionary interactions between natural populations of plants and herbivores has grown considerably since Ehrlich and Raven (1964) drew attention to the concept of coevolution by emphasizing its explanatory power regarding a variety of important ecological problems. Early studies in this area

focused on intricately woven interactions (Gilbert 1971, Wiebes 1979) that may have resulted from coevolutionary events that occurred on a geological time scale. Recently, research approaches in this area have expanded to include investigation of the impact of short term (1-100 years) coevolutionary processes on population dynamics and community level interactions of plants and herbivores in natural systems. Information gained from these basic studies is at least in part transferable to agricultural systems where the time scale and questions are similar.

Recent studies of resistance of wild plant populations to insect and microbial herbivores (see Berenbaum and Zangerl 1987, Burdon 1980, Gould 1983, for reviews), and of herbivore adaptations to plants (see Gould 1983, Mitter and Futuyma 1983, Papaj and Rausher 1983, Parker 1985) indicate that, within species, genetic variation in both traits is common. Some studies have revealed genetic variation in plant resistance and in herbivore adaptation on a local scale as small as one square mile. Given these data along with recent evidence that herbivores often have a significant impact on growth and reproduction of native plants (Augsberger 1984, Bentley and Whittaker 1979, Louda 1984, Morrow and LaMarche 1978, Parker and Root 1981, Rausher 1981), the stage is set for ecological-genetic interactions in which herbivores affect the freqency of plant genes conferring resistance. In turn, these resistant plants may reciprocally affect the frequency of herbivore genes conferring an ability to overcome the defense. One expected consequence of this evolutionary interaction is for the fitness and population size of plant and herbivore species to be influenced by which organisms adapt most quickly and dramatically.

EVOLUTION IN NATURAL VS. AGRICULTURAL SYSTEMS

In agricultural systems, we often short-circuit the natural coevolutionary process by imposing artificial selection regimes on our crop plants (ie, plant breeding). In many cases plant breeding efforts inadvertently select for crops with heightened pest susceptibility (dePonti 1981, Harlan 1976), while specially designed breeding programs cause crops to acquire genetic resistance to pests at a much faster rate than that expected due to natural selection by herbivores (Gould 1983). While our breeding programs may disrupt the operation of natural selection on our crops, we cannot halt this adaptive process in crop pests. Indeed, case studies have found microbial and insect herbivores becoming totally adapted to resistant crops after 2-7 years of their use on a commercial scale (Johnson 1983, Kiyosawa 1982, Sosa 1978).

Plant pathologists and entomologists who have made general comparisons of pathogen and insect evolution in natural and agricultural systems have suggested that adaptation to resistant crops may be more rapid than adaptation to resistance in natural plant populations (Alexander 1984, Ankister and Wahl 1979, Browning 1980, Burdon 1980, Dinoor and Eshed 1984, Oates et al. 1983). They believe that the genetic homogeneity of crops compared to wild plants is one of the important factors (Barrett 1981). Thus, the larger the acreage planted to a single resistant crop cultivar, the more rapid will be pest adaptation. If this is true, current economic and technical constraints on the classical breeding of resistant crops may be a blessing in disguise. Because these constraints have limited our ability to produce high yielding, popular cultivars that also have high levels of pest resistance, the acreage planted to resistant cultivars has been limited. This restriction on acreage may, in turn, be slowing the pace at which pests adapt to many currently marketed cultivars.

One of the goals of genetic engineering is removal of some technical and economic constraints of classical plant breeding (Comai and Stalker 1984). With these constraints removed, there would be economic pressure to develop and plant more acreage with pest-resistant crops. One could envision the long-term (50-100 years) consequences of unbridled success in this effort to be devastating, as pests adapt to huge monocultures of resistant crops. If instead of boldly unleashing our new technology, we utilize knowledge about herbivore adaptation in natural plant communities (and more information that is forthcoming, e.g Alexander 1987), the tools of genetic engineers may be used to produce more sophisticated crop cultivars that could delay or beneficially manipulate adaptation by pests in ways that were previously inconceivable.

EVOLUTIONARILY INSURMOUNTABLE DEFENSES?

Investigations of natural ecosystems, and our experience with classically bred resistant crops, suggest approaches to the engineering of resistant plants that range from simple to complex and counter intuitive. One straight forward approach that has been applied to the problem of pest adaptation to resistant cultivars has been searching for plant defenses to which herbivores have little or no genetic potential for adaptation. Such defenses are likely to yield evolutionarily stable benefits to the plant lineages producing them no matter how abundantly and widely they are planted. These insurmountable defenses are likely to be found in the dreams of plant breeders whose worst fear is spending half a career using classical breeding techniques to develop a resistant cultivar that is genetically overcome by the pest after less than 10 years of commercial use. This concern has motivated plant breeders to search for ways to predict the intrinsic insurmountability of pest resistance characteristics before

they are transferred from wild crop relatives or agronomically inferior cultivars to modern cultivars.

To date, the best predictor of resistance factors that may have intrinsic immunity to microbial pest adaptation has been the number of genes coding for variation in expression of the resistance trait(s). Empirical evidence with pathogen-resistant cultivars supports the concept that cultivars whose resistance is determined by many genes (polygenic) are often more durable in the field than those in which resistance is determined by a single gene (monogenic) (Johnson 1983). Exceptions to this prediction do occur (Johnson 1983, Parlevliet 1983), but the greatest stumbling block to this approach has been the fact that it usually takes more effort for a classical plant breeding team to move a polygenic resistance trait into a cultivar than a monogenic trait. From a genetic engineering perspective, we currently lack the technology required for transferring polygenically controlled traits.

Coevolutionary theorists have also been interested in evolutionarily insurmountable defenses and have offered some alternative approaches for predicting immunity of a resistance factor to herbivore adaptation (Feeny 1975, 1976, Rhodes and Cates 1976). Simply stated, resistance factors found in plant species or plant tissues that are most consistently exposed to herbivore damage are likely to be most immune to herbivore adaptation, or their effectiveness would have been lost thousands of years ago. This concept has been useful, although the difficulty of judging the actual degree of exposure of a plant species to herbivores has caused confusion. Additionally, empirical findings have not supported some predictions of this concept (Berenbaum 1980, Bernays 1978).

Because many herbivore species in natural communities feed on or attack plant species that are taxonomically closely related, it has been suggested that the toxic chemicals in unrelated plants are so novel to the herbivores that they often preclude adaptation (e.g. Ehrlich and Raven 1964). This idea would be welcomed by genetic engineers who can transfer genes among unrelated plants, and even unrelated phyla. Unfortunately, the strength of this hypothesis is diminished by data on the taxonomically diverse hosts of polyphagous (and some oligophagous) herbivores. The rapid adaptation of herbivores to the totally novel chemical structures of some synthetic pesticides adds further to concern about the generality of this hypothesis. Although it would be foolish to totally abandon the search for resistance factors with some intrinsic immunity to pest adaptation, this approach doesn't seem to be very promising.

FACTORS GOVERNING THE RATE AND MODE OF HERBIVORE ADAPTATION

The question of how to predict which chemically based resistance factors in natural systems are likely to possess intrinsic immunity to herbivore adaptation has been changing to a question of whether there are a significant number of such resistance factors. Almost all recent studies of natural plant/herbivore interactions have found significant genetic variation within and between herbivore populations for ability to tolerate plant resistance factors (see Gould 1983, Mitter and Futuyma 1983, Parker 1985). But, if most herbivores in natural systems have the genetic variation necessary to adapt to plant defenses, what inhibits them from achieving this adaptation?

As with most population genetic processes, factors expected to influence the rate of herbivore adaptation are; selection intensity, initial frequencies of adaptive

alleles, dominance, epistasis, and the ecological and physiological costs of adaptation. Empirical studies indicate that there is a high degree of variation in these parameters from one plant/herbivore system to another (Gould 1983,1987). For example, there are cases where plant resistance causes herbivore individuals lacking adaptive alleles at one gene locus to have close to 0% expected survivorship, while average survival of individuals with two adaptive alleles at that locus is almost 100% (barring factors unrelated to nutrition (Gallun and Hatchett 1969)). In other cases, a plant trait may confer resistance to fitness reduction by herbivores but have an insignificant effect on the herbivore's fitness (Gould 1983).

Generally we can assume that the less a plant resistance factor reduces herbivore fitness, the slower will be the rate at which herbivores adapt to the resistance factor. Thus, among the dreams of plant breeders is a resistance factor controlled by one gene, that allows a cultivar to withstand high pest populations while causing little or no reduction in pest fitness. A few crop cultivars exist that approach this idealized state. These "tolerant" cultivars do not reduce herbivore feeding or survival, but are less affected than related cultivars by the loss of some vegetative or reproductive tissues (Mussell 1980, Painter 1951). Other types of resistance factors can cause a shift in pest feeding away from plant structures such as young fruits that are often important to yield. The pests wind up feeding on less important structures such as older leaves. These cultivars may cause only slight reduction in herbivore fitness. (The relationship of pest fitness in one crop to the season long population dynamics of polyphagous pests is often complex. In these cases the appropriateness of tolerant cultivars must be assessed on an individual cropping system basis (Kennedy et al. 1987)).

Intensity of selection pressure for herbivore adaptation involves more than the affect of a resistant plant on the herbivorous individuals that feed on it. To assess the selection pressure caused by a plant resistance factor, we must assess its effect within the context of the entire herbivore population. If a resistance factor causes 100% mortality of herbivorous individuals that feed on it, but plants with this factor account for only 1% of an herbivore population's food, selection pressure for herbivore adaptation is minimal. If a resistance factor causes an 80% decrease in herbivore fitness and is present in 100% of the plants that one herbivore population feeds on, but is only present in 50% of a second herbivore population's hosts, the resitance factor will cause twice the decrease in average herbivore fitness in population 1 as the same resistance factor will in herbivore population 2. Therefore, it might seem logical that herbivores in the second population will adapt half as rapidly as the herbivores in the first population. In fact, this is not expected in sexual species according to population genetic theory. Even though the herbivore fitness reduction in these two situations differs by only a factor of 2, the difference in the rate of herbivore adaptation in the two situations may be greater than this amount.

Figure 1 illustrates the relationship between the abundance of the resistant plants relative to other food resources, and the rate of herbivore adaptation. The genetic model used to generate Figure 1 assumes that herbivore adaptation to the defense involves a single gene locus with two alleles (Gould 1986a). If the heterozygous individuals have a mortality rate that is linearly intermediate between the two homozygotes, the rate of adaptation to the resistance factor in population 1 is predicted to be approximately three times the rate of adaptation in population 2 (Fig 1a). If the adaptive alleles are recessive

to the non-adaptive alleles population 1 adapts five times faster than population 2 (Fig 1b).

In the case of Hessian fly resistant wheat where resistant plants can cause 90-100% mortality of fly larvae, alleles at one locus appear responsible for fly adaptation to single resistance genes in the wheat and the adaptive alleles seem to be recessive (Gould 1986b). Both theory and field experience over the past twenty years indicate that when most of the Hessian fly population (>80%) is exposed to resistant plants, the fly population will rapidly adapt to resistant wheat (less than ten years; Gallun and Khush 1980, Gould 1986b). Genetic models predict that if fly populations were exposed to a seed mixture of one half resistant and one half susceptible plant genotypes they would take much longer to adapt (Gould 1986b). In a crop like wheat in which some yield losses due to damaged plants are compensated for by increased growth and yield of undamaged neighboring plants, growing mixtures of resistant and susceptible plants in the same fields could offer reasonable crop protection for an extended period of time. Unfortunately, due to agronomic and marketing considerations, this approach would be feasible only if we have the technology for efficiently developing wheat cultivars with similar harvesting and milling characteristics, but different resistance traits. Current breeding techniques can produce such cultivars in some crops but not in others. And, even in cases where such cultivars can be developed, the extra effort required is significant. In the future, plant genetic engineering techniques could turn this difficult problem into a simpler task.

FIGHT OR FLIGHT

So far I have discussed only one way in which pests can adapt to resistant plants, ie. by physiological mechanisms. Another adaptive pathway available to insects is behavioral

avoidance of the resistant plants. Studies of insect herbivore genetics reveal that these animals are at least as genetically variable in terms of host selection as they are in physiological adaptability (Jaenike 1985, Tabashnik et al. 1981, Wasserman and Futuyma 1981). If a pest such as the cotton bollworm (Heliothis zea) that feeds on cotton and soybean is faced with a huge acreage of resistant cotton that causes 80% mortality, will it adapt physiologically and nullify the utility of the resistant cotton germplasm, or will it genetically shift its host preference to soybean where its feeding causes less yield reduction and where it is more easily controlled? Conditional answers to this intricate problem have been offered by a number of recent genetic models (Futuyma 1987, Gould 1984, Rausher unpubl. results). In general, the combined use of genes coding for a toxin and genes coding for repellency will offer longer lasting resistance to polyphagous pests than either one alone. In some cases such combined factors are predicted to offer almost infinitely stable resistance to the pest (Gould 1983, Kennedy et al. 1987).

For less polyphagous insects the results are more difficult to predict. According to the genetic models some of the important ecological-genetic factors determining the outcome are 1) initial allele frequencies of genes for avoidance and for physiological adaptation 2) mode of population regulation, and 3) ecological and physiological costs of adaptation to the resistant crop. It is easy to get lost in the detailed interactions mimicked by these models, but even qualitative information on one of the above parameters could be useful in predicting how a specific pest will respond to incorporation of a resistance factor into a crop. If genetic engineering techniques give us the flexibility to pick and choose among the resistance factors we put in our crops, these genetic models offer suggestions regarding the best combinations to use.

MIXING YOUR POISONS

Genes for repellancy to a specific insect may not be available in some agricultural systems and are never an option for pathogen control. In these situations the choice between incorporating one, two, or more toxins into a single cultivar can be subjected to analysis regarding probable evolutionary stability. Genetic models addressing this problem indicate that widespread planting of a cultivar with two resistance factors may in some cases offer very good insect control initially, but will be adapted to faster than a cultivar with a single resistance factor because of the intense selection pressure the combined resistance factors would exert (Gould 1986a). These same models indicate that if 80% of the plants in a field contained two toxic resistance factors while 20% contained no resistance factors, pests are likely to adapt much more slowly (Gould 1986a). In the specific case of the Hessian fly, where we have a reasonably good understanding of the pest's genetics , it would probably take the flies 10-40 times as long to adapt to the 80/20 mixture as it would take them to adapt to a widely used cultivar in which all plants contained one toxic resistance factor (Gould 1986b).

At this point it is difficult to tell just how much flexibility will be gained from developments in genetic engineering. However, even with the greatest achievements conceived of to date, it will still be easier to develop genetically homogeneous cultivars than mixtures. Long-term stability of yield is not expected to be (nor should it be expected to be) of primary concern to private agricultural enterprises. Their orientation toward relatively short-term profit is likely to lead to production of only the most simple resistant cultivars. Additionally, open market competition may lead to advertising aimed at convincing the

farmer that the cultivar with the highest percentage of very toxic plants will offer the greatest profits.

There may be optimists who believe genetic engineering will offer such an unlimited supply of new resistance factors that concern over evolutionary stability is unwarranted. When one resistance factor becomes obsolete due to pest evolution, the engineer will merely slip in a new resistance factor. This is a possible scenario, but not one worthy of too much faith before we know the limits of biotechnology.

ECOLOGICAL TRADE-OFFS

Another optimistic view may be extracted from the old ecological paradigm "a jack of all trades is a master of none", the implication being that as an herbivore adapts to one plant defense, its physiological efficiency in dealing with other plant defenses will decrease. This adage has been used to explain the prevalence of ecologically specialized plant and animal species, including herbivores that feed on only one or a few host plant species (eg. MacArthur 1972). In some cases tests have lent support to this hypothesis (eg. Lewis 1986); but, in terms of herbivore host utilization, some empirical tests have shown that insect herbivore species that feed on an array of plant groups are as physiologically efficient as specialists (eg. Futuyma and Wasserman 1981, Scriber 1983).

More detailed genetic studies searching for tradeoffs between a specific genotype's adaptedness to one host and its fitness on another host have often found single herbivore genotypes that perform best on both hosts (Rausher 1984, Service 1984, Via 1984). Laboratory selection experiments have found cases in which herbivore strains selected for adaptation to one plant species or one chemical defense of a plant, perform relatively poorly when reared on

another host plant or when exposed to another type of defensive chemical. However, in more cases, the strains selected for adaptation to one resistance factor seem to become cross-adapted to other resistance factors. For example, an herbivorous mite species selected for physiological adaptation to a resistant cucumber cultivar became more adapted to potato and tobacco than the control-strain from which it was derived (Gould 1979). Weber (1985) tested over 1000 clones of an aphid species (Myzus persicae) for population growth rate on sugar beets and tomato. He found no possitive or negative correlation between the clones' growth rates on these two taxonomically distinct hosts. Plant pathologists have also addressed the question of ecological costs involved in adaptation to resistant plants. In some cases they have found an ecological cost associated with microbial adaptation to resistant crop cultivars, but in most cases these costs are small or non-existent (Leonard 1986, Parlevliet 1983).

This apparent lack of a general or predictable ecological tradeoff in herbivore adaptation to taxonomically and chemically diverse host plants requires careful consideration. In some cases, such as Hessian fly adaptation to wheat, genes responsible for adaptation appear to be very specific to the resistance factor causing the selection pressure. However, as demonstrated by some of the examples above, adaptation of herbivores to plant resistance factors may be very general. A plant breeder can find enough material for nightmares just worrying about specific herbivore adaptations. When we realize that planting a large acreage of one herbivore-resistant cultivar could generate cross adaptation to other crop species, there is reason for broader concern. Perhaps most pest adaptations will be very specific, but if evolution of adaptation to insecticides is any guide, cross-resistance to toxic chemicals is expected in field populations (Georghiou 1986).

GENETIC ENGINEERING AND IPM

One of the most broad ranging side effects of successful engineering of uniformly resistant crops could be the elimination of integrated pest management (IPM) techniques that aim at using pest suppressive measures only when pests are likely to cause economically important damage. In IPM programs, field scouts are used to determine if enough pests are present to warrant the use of a pesticide treatment. This approach generally minimizes the intensity and frequency of selective pressure for adaptation to the pesticide. Since pesticides may usually be purchased a week or less before use and can be stored for long periods of time, the farmer has a high degree of flexibility regarding IPM decisions. This is not the case with resistant seed that must be purchased at least a month or two before the pest's abundance can be judged. If resistant seed is not too expensive and yields well, farmers are likely to buy it every year as "insurance" against a pest outbreak. The problem here is that the resistant crop will cause the same selection pressure for pest adaptation whether the density of the pest is high or low. Some pests that outbreak sporadically may adapt to a resistant cultivar before it has ever been useful in significantly reducing yield loss.

Unless genetic engineers develop cultivars that produce toxins only when high levels of pest attack are expected, U.S. agriculture may be thrown back to the prophylactic pest suppression tactics used in the DDT era. Production of conditionally toxic plants is one technically complex approach to this problem that is at least biologically feasible. Recent investigations have found that some wild plants and crops can respond to pest damage by increasing toxin production in relation to pest damage levels (Carroll and Hoffman 1980, Tallamy 1985). Progress is

also being made in the discovery of natural and synthetic chemicals that induce plants to produce resistance factors (Sequeira 1980, Ouchi 1983). If these chemicals act at low doses and are not prohibitively expensive to produce they may lead to the development of resistance factors compatible with integrated pest management approaches.

Another tactic for retarding pest adaptation involves engineering plants to produce toxins in specific tissues at specific times of the year. Such specificity in toxin production is commonly found in plants. For example, alkaloids are generally found in higher concentrations in young compared to mature leaves (McKey 1979). It may therefore be possible to regulate the tissue specific expression of toxin coding genes that are engineered into crops.

As a concrete example of how genetic engineering and IPM can be interfaced it is useful to consider tobacco plants that have been engineered to produce a toxin of bacterial origin. Mechanical applications of the bacteria, Bacillus thuringiensis (B.t.) have been used for many years as a minor or major component of IPM programs in agriculture and forestry. Although resistance to the toxin produced by B.t. has rarely been found in the field, insects can adapt to it (McGaughey 1985). Rohm and Haas Company has been granted permission to field test tobacco that produces B.t. toxin (Crawford 1986), so it is worth considering how best to develop commercial tobacco cultivars with current biotechnological methods. The major target pests for B.t. toxin are the tobacco budworm and tobacco hornworm. Each of these pests cause some economic damage in most years, but require little control effort in many years. Each pest has three or more generations per year in the major tobacco growing areas, but cause most of their damage in one of these generations. Deployment of a tobacco cultivar that

consistently produces high levels of the B.t. toxin is likely to cause adaptations to evolve in the pests. Development of tobacco cultivars that only produce B.t. toxin during the pest generation when economic damage is expected, could reduce selection for pest adaptation. The development of cultivars that only produce B.t. toxin in leaf-bud tissue could reduce the major form of tobacco budworm damage while decreasing the average selection pressure on the budworm population. Given that nicotine concentration in tobacco plants varies more than an order of magnitude during the growing season, and also varies based on leaf age (Darkis et al. 1936,1952) similar regulation of other toxin levels in tobacco cultivars is not inconceivable.

In addition to damaging tobacco, the tobacco budworm and tobacco hornworm damage cotton and tomato respectively. It may become economically feasible to use mechanically applied B.t. for control in these crops, so any adaptation to B.t. caused by widespread use of B.t. toxin-producing tobacco could impact future IPM efforts in cotton and tomato. If genes for B.t. toxin production were incorporated into cotton and tomato as well as tobacco, we could anticipate an even more rapid loss of B.t. as a biological, IPM tool. Rohm and Haas Co. may be unaware of the problems that may develop from their intentions to transfer B.t.- toxin genes to "crop plants ranging from citrus to rice" (Crawford 1986).

REGULATING AND GUIDING THE USE OF RESISTANCE GENES

9 A number of authors have presented cases for considering crop germplasm (ie. genes) as a natural resource (Frankel and Hawkes 1975, NAS 1978). It has been argued that any action that carelessly endangers our germplasm bank could be viewed as an infraction of federal regulations (Sun 1986). Although defining what is involved in being careless

is difficult, the issue is worthy of detailed consideration. The major concerns up to now have been with the way we manage genetic information (mostly exotic seed) housed in storage centers and with inadequate programs for further collection of exotic genetic material (Shulman 1986, Sun 1986). This article has outlined one more area of concern when dealing with genes for pest resistance. Genetic resources in germplasm banks that may be used to develop drought resistant crops in the future, face the threat of being lost through neglect of seed viabilty and other operations required for saving this genetic information from extinction (Shulman 1986, Sun 1986). Genetic resources in germplasm banks that code for production of pest resistance factors also face this threat but additionally face the threat of misuse.

As in natural systems, any genetic lineage that possesses genes for drought adaptation or adaptation to other abiotic factors will always be adapted to these factors. This is not the case with genetic adaptations to biotic environmental factors such as herbivores, that can evolve. The wise use of genetic engineering in this area could lead to higher and more stable crop yields. Its overuse or abuse could cause the loss of valuable resources.

To do this, it seems important for government and industry to sponsor basic research aimed at enhancing our understanding of the genetic and environmental regulation of plant toxin production as well as research on the genetics of plant/herbivore interactions in natural and agricultural systems. Incentives from government to stimulate breeders in land grant schools and industry to develop efficient methods for producing genetically heterogenious crops will be essential in this effort aimed at long-term gains.

Given that the field of genetic engineering is still in its infancy, the 1980's may be the best time for developing fruitful interactions between industry, academia, and government. Although rigid regulations on the biotechnology industry could be stifling, academia and government may be able to guide the development of genetically engineered crops in creative ways that would lead to economically feasible, development of stable crop resistance.

FIGURE CAPTIONS

Figure 1

Factors governing the rate of physiological adaptation to a resistant crop by a sexually reproducing herbivore. Resistant plants cause an 80% reduction in survival of homozygous, unadapted individuals. In figure 1A adaptation is inherited additively. In 1B adaptation is inherited recessively. The (—△—) line represents the situation where all plants that the herbivore population utilizes carry the resistance producing genes. The (—□—) line represents the situation in which 50% of the plants are

References Cited

Alexander, H. M. 1984. Spatial patterns of disease induced by Fusarium moniliforme var. subglutinans in a population of Plantago lanceolata. Oecologia (Berl.) 62: 141-143.

Alexander, H. M. 1987. Spatial heterogeneity and disease in natural populations. In ,ed. Plant Epidemiology. [In press]

Ankister, Y., and I. Wahl. 1979. Coevolution of the rust fungi on Gramineae and Liliaceae and their hosts. Annu. Rev. Phytopathol. 17: 367-403.

Augspurger, C. K. 1984. Seedling survival of tropical tree species: interactions of dispersal distance, light-gaps, and pathogens. Ecology 65: 1705-1712.

Barrett, J. A. 1981. The evolutionary consequences of monoculture. Pages 209-248 in J. A. Bishop and L. M. Cook, eds. Genetic Consequences of Man-made Change. Academic Press, London.

Bentley, S., and J. B. Whittaker. 1979. Effects of grazing by a chrysomelid beetle, Gastrophysa viridula on competition between Rumex obtusifolius and Rumex crispus. J. Ecol. 67: 79-90.

Berenbaum, M. R. 1980. Adaptive significance of midgut pH in larval Lepidoptera. Amer. Natur. 115: 138-146.

Berenbaum, M. R., and A. R. Zangerl. 1987. Stalemates in the coevolutionary arms race: syntheses, synergisms, and sundry other sins. In K. Spencer, ed. Chemically Mediated Coevolution. Pergamon Press, New York. [In press]

Bernays, E. A. 1978. Tannins: an alternative viewpoint. Ent. Exp. Appl. 24: 44-53.

Browning, J. A. 1980. Genetic protective mechanisms of plant-pathogen populations: their coevolution and use in breeding for resistance. Pages 52-75 in M. K. Harris, ed. Biology and Breeding for Resistance to Arthropods and Pathogens in Agricultural Plants. Texas Agric. Exp. Stn., Texas A&M University, College Station.

Burdon, J. J. 1980. Variation in disease-resistance within a population of Trifolium repens. J. Ecol. 68: 737-744.

Carroll, C. R., and C. A. Hoffman. 1980. Chemical feeding deterrent mobilized in response to insect herbivory and counter adaptation by Epilachna tredecimnotata. Science 209: 414-416.

Comai, L., and D. M. Stalker. 1984. Impact of genetic engineering on crop protection. Crop Protec. 3: 399-408.

Crawford, M. 1986. Test of tobacco containing bacterial gene approved. Science 233: 1147.

Darkis, F. R., L. F. Dixon, F. A. Wolf, and P. M. Gross. 1936. Flue-cured tobacco: correlation between chemical composition and stalk position of tobacco produced under varying weather conditions. J. Ind. Engin. Chem. 28: 1214-1223.

Darkis, F. R., L. A. Baisden, P. M. Gross, and F. A. Wolf. 1952. Flue-cured tobacco: chemical composition of leaf and blade tissue. J. Ind. Engin. Chem. 44: 297-305.

dePonti, O. M. B. 1981. Conserving the natural resistance to insects by a proper use of the pesticide umbrella. Pages 236-248 in Proceedings, 19th Eucarpia Congress, Leningrad.

Dinoor, A., and N. Eshed. 1984. The role and importance of pathogens in natural plant communities. Annu. Rev. Phytopathol. 22: 443-466.

Ehrlich, P. R., and P. H. Raven. 1964. Butterflies and plants: a study in coevolution. Evolution 18: 586-608.

Feeny, P. P. 1975. Biochemical coevolution between plants and their insect herbivores. Pages 3-19 in L. E. Gilbert and P. R. Raven eds. Coevolution of Animals and Plants. University of Texas Press, Austin.

Feeny, P. P. 1976. Plant apparency and chemical defense. In J. Wallace and R. Mansell, eds. Biochemical Interactions Between Plants and Insects. Rec. Adv. Phytochem. 10: 1-40.

Frankel, O. H., and J. G. Hawkes. 1975. Crop Genetic Resources for Today and Tomorrow. Cambridge University Press, Cambridge.

Futuyma, D. J. 1983. Evolutionary interactions among herbivorous insects and plants. Pages 207-231 in D. J. Futuyma and M. Slatkin, eds. Coevolution. Sinauer Associates, Sunderland, MA.

Futuyma, D. J. 1987. The role of behavior in host-associated divergence in herbivorous insects. In M. D. Huettel, ed. Evolutionary Genetics of Invertebrate Behavior. Plenum Press, New York. [In press]

Futuyma, D. J., and S. S. Wasserman. 1981. Food plant specialization and feeding efficiency in the tent caterpillars, Malacorsoma disstria Hubner and M. americanum (F.). Ent. Exp. Appl. 30: 106-110.

Gallun, R. L., and J. H. Hatchett. 1969. Genetic evidence of elimination of chromosomes in the Hessian fly. Ann. Entomol. Soc. Am. 62: 1095-1101.

Gallun, R. L., and G. S. Khush. 1980. Genetic factors affecting expression and stability of resistance. Pages 64-85 in F. G. Maxwell and P. R. Jennings, eds. Breeding Plants Resistant to Insects. Wiley & Sons, New York.

Georghiou, G. P. 1986. The magnitude of the resistance problem. Pages 14-43 in E. H. Glass (Chairman). Pesticide Resistance Strategies and Tactics for Management. National Academy Press, Washington, D.C.

Gilbert, L. E. 1971. Butterfly-plant coevolution: has Passiflora adenopoda won the selectional race with Heliconiine butterflies? Science 172: 585-586.

Gillett, J. W., A. M. Stern, S. A. Levin, M. A. Harwell, M. Alexander, and D. A. Andow. 1985. Potential Impacts of Environmental Release of Biotechnology Products: Assessment, Regulation, and Research Needs. Ecosystems Research Center, Cornell University, Ithaca.

Gould, F. 1979. Rapid host range evolution in a population of the phytophagous mite, Tetranychus urticae Koch. Evolution 33: 791-802.

Gould, F. 1983. Genetics of plant-herbivore systems: interactions between applied and basic study. Pages 599-653 in R. Denno and B. McClure, eds. Variable Plants and Herbivores in Natural and Managed Systems. Academic Press, New York.

Gould, F. 1984. Role of behavior in the evolution of insect adaptation to insecticides and resistant host plants. Bull. Entomol. Soc. Am. 30: 33-41.

Gould, F. 1986a. Simulation models for predicting durability of insect-resistant germ plasm: a deterministic diploid, two-locus model. Environ. Entomol. 15: 1-10.

Gould, F. 1986b. Simulation models for predicting durability of insect-resistant germ plasm: Hessian fly (Diptera: Cecidomyiidae)-resistant winter wheat. Environ. Entomol. 15: 11-23.

Gould, F. 1987. Genetics of chemically-mediated coevolution of plant/herbivore interactions, Pages in K. Spencer, ed. Chemically Mediated Coevolution.

Hardy, R. W. 1986. Biotechnology in pesticide resistance development. Pages 130-141 in E. H. Glass, Chairman. Pesticide Resistance. Strategies and Tactics for Management. National Academy Press, Washington, D.C.

Harlan, J. R. 1976. Diseases as a factor in plant evolution. Annu. Rev. Phytopathol. 14: 31-51.

Jaenike, J. 1985. Genetic and environmental determinants of food preference in Drosophila tripunctata. Evolution 39: 362-369.

Johnson, R. 1983. Genetic background of durable resistance. Pages 5-26 in F. Lamberti, J. M. Waller, and N. A. Van der Graaf, eds. Durable Resistance in Crops. Plenum Press, New York.

Kennedy, G. G., F. Gould, O. M. B. dePonti, and R. E. Stinner. 1987. Ecological, agricultural, genetic, and commercial considerations in the deployment of insect-resistant germ plasm. Environ. Entomol. (In press)

Kiyosawa, S. 1982. Genetics and epidemiological modeling of breakdown of plant disease resistance. Annu. Rev. Phytopathol. 20: 93-117.

Kolata, G. 1985. How safe are engineered organisms? Science 229: 34-35.

Leonard, K. J. 1986. The host population as a selective factor. Pages 163-179 in M. S. Wolfe and C. E. Caten, eds. Populations of Plant Pathogens: Their Dynamics and Genetics. Blackwell, Oxford.

Leonard, K. J., and R. J. Czochor. 1980. Theory of genetic interactions among populations of plants and their pathogens. Annu. Rev. Phytopathol. 18: 237-258.

Lewis, A. C. 1986. Memory constraints and flower choice in Pieris rapae. Science 232: 863-865.

Louda, S. M. 1984. Herbivore effect on stature, fruiting, and leaf dynamics of a native crucifer. Ecology 65: 1379-1386.

MacArthur, R. H. 1972. Geographical Ecology. Harper & Row, New York.

Maxwell, F. G., and P. R. Jennings. 1980. Breeding Plants Resistance to Insects. Wiley & Sons, New York.

McGaughey, W. H. 1985. Insect resistance to the biological insecticide Bacillus thuringiensis. Science 229: 193-194.

McKey, D. 1979. The distribution of secondary compounds within plants. Pages 56-133 in G. A. Rosenthal and D. H. Janzen, eds. Herbivores: Their Interactions with Secondary Plant Compounds. Academic Press, New York.

Mitter, C., and D. J. Futuyma. 1983. An evolutionary-genetic view of host plant utilization by insects. Pages 427-459 R. F. Denno and M. S. McClure, eds. Herbivores and Their Variable Hosts in Natural and Managed Systems. Academic Press, New York.

Morrow, P. A., and V. C. LaMarche. 1978. Tree ring evidence for chronic insect suppression of productivity in subalpine Eucalyptus. Science 201: 1244-1246.

Mussell, H. 1980. Tolerance to disease. Pages 39-52 in J. G. Horsfall and E. B. Cowling, eds. Plant Diseases: An Advanced Treatise, Vol. 5. Academic Press, New York.

N.A.S. 1978. Conservation of germplasm resources: an imperative. N.A.S., Washington, D.C.

Oates, J. D., J. J. Burdon, and J. B. Brouwer. 1983. Interactions between Avena and Puccinia species. II. The pathogens: Puccinia coronata CDA and P. graminis Pers. F. sp. avenae Eriks. & Henn. J. Appl. Ecol. 20: 585-596.

Ouchi, S. 1983. Induction of resistance or susceptibility. Annu. Rev. Phytopathol. 21: 289-315.

Painter, R. H. 1951. Insect Resistance in Crop Plants. Macmillan Press, New York.

Papaj, D. R., and M. D. Rausher. 1983. Individual variation in host location by phytophagous insects. Pages 77-124 in S. Ahmad, ed. Herbivorous Insects: Host-seeking Behavior and Mechanisms. Academic Press, New York. 257 pp.

Parker, M. A. 1985. Local population differentiation for compatibility in an annual legume and its host-specific fungal pathogen. Evolution 39: 713-723.

Parker, M. A., and R. B. Root. 1981. Insect herbivores limit habitat distribution of a native composite, Machaeranthera canescens. Ecology 62: 1390-1392.

Parlevliet, J. E. 1983. Models explaining the specificity and durability of host resistance derived from the observations on the barley-Puccinia hordei system. Pages 57-80 in F. Lamberti, J. M. Waller, and N. A. Van der Graaf, eds. Durable Resistance in Crops. Plenum Press, New York.

Rathcke, B. J., and R. W. Poole. 1975. Coevolutionary race continues: butterfly larval adaptation to plant trichomes. Science 187: 175-176.

Rausher, M. D. 1981. Host plant selection by Battus philenor butterflies: the roles of predation, nutrition, and plant chemistry. Ecol. Monogr. 51: 1-20.

Rausher, M. D. 1984. Tradeoffs in performance on different hosts: evidence from within- and between-site variation in the beetle Deloyala guttata. Evolution 38: 582-595.

Rhoades, D. F., and R. G. Cates. 1976. Toward a general theory of plant antiherbivore chemistry. Pages 168-213 in J. W. Wallace and R. L. Mansell, eds. Biochemical Interaction Between Plants and Insects. Plenum Press, New York.

Rosenthal, G. A., and D. H. Janzen. 1979. Herbivores. Their Interaction With Secondary Plant Metabolites. Academic Press, New York.

Russell, G. E. 1978. Plant Breeding for Pest and Disease Resistance. Butterworths, London.

Scriber, J. M. 1983. The evolution of feeding specialization, physiological efficiency and host races in selected Papilionidae and Saturniidae, Pages in R. F. Denno and M. S. McClure, eds. Impact of Variable Host Quality on Herbivorous Insects. Academic Press, New York.

Service, P. 1984. Genotypic interactions in an aphid-host plant relationship: Uroleucon rudbeckiae and Rudbeckia lacinata. Oecologia (Berl.) 61: 271-276.

Sequeira, L. 1980. Defenses triggered by the invader: recognition and compatibility phenomena. Pages 179-200 in J. G. Horsfall and E. B. Cowling, eds. Plant Disease: An Advanced Treatise, Vol. 5. Academic Press, New York.

Sharples, F. E. 1982. Spread of organisms with novel genotypes: thoughts from an ecological perspective. ORNL/TM-8473. Oak Ridge National Laboratory Environmental Sciences Division, Publication 2040, Oak Ridge, TN.

Shulman, S. 1986. Seeds of controversy. BioScience 36: 647-651.

Sosa, O. 1978. Biotype L, ninth biotype of the Hessian fly. J. Econ. Entomol. 71: 458-460.

Sun, M. 1986. Fiscal neglect breeds problems for seed banks. Science 231: 329-330.

Tabashnik, B. E., H. Wheelock, J. D. Rainbolt, and W. B. Watt. 1981. Individual variation in oviposition preference in the butterfly, Colias eurytheme. Oecologia (Berl.) 50: 225-230.

Tallamy, D. W. 1985. Squash beetle feeding behavior: an adaptation against induced cucurbit defenses. Ecology 66: 1574-1579.

Tangley, L. 1986. Agricultural biotechnology: who's holding the reins? BioScience 36: 652-655.

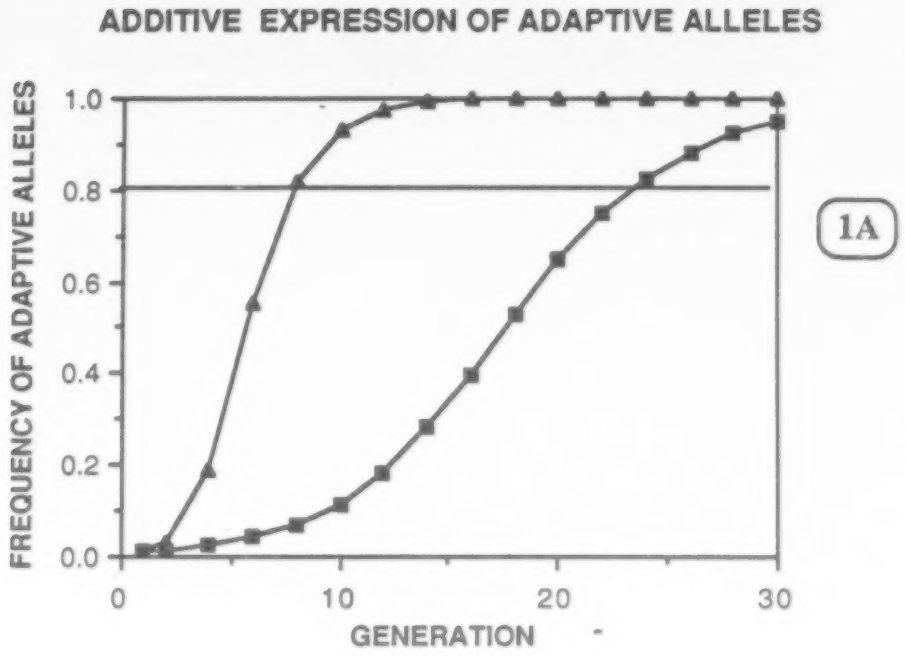
Via, S. 1984. The quantitative genetics of polyphagy in an insect herbivore, I. Genotype-environment interaction in larval performance on different host plant species. Evolution 38: 881-895.

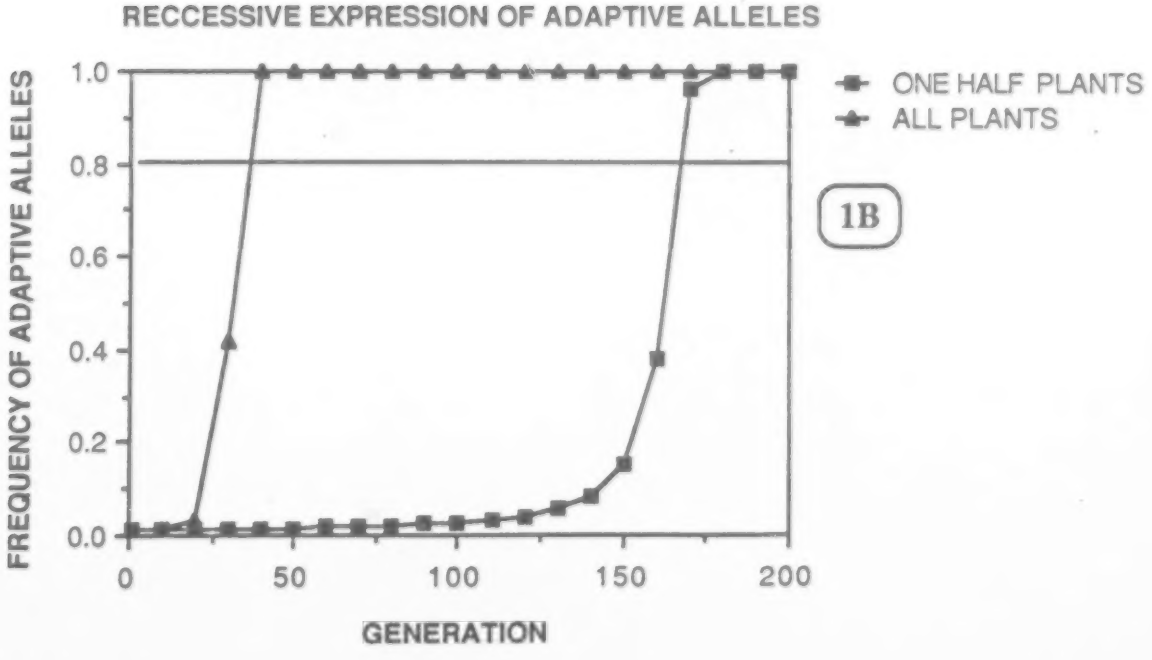
Wasserman, S. S., and D. J. Futuyma. 1981. Evolution of host plant utilization in laboratory populations of the southern cowpea weevil, Callosobruchus maculatus Fabricius (Coleoptera: Bruchidae). Evolution 35: 605-617.

Weber, G. 1985. Genetic variability in host plant adaptation of the green peach aphid, Myzus persicae. Entomol. Exp. Appl. 38: 49-56.

Wiebes, J. J. 1979. Co-evolution of figs and their insect pollinators. Annu. Rev. Ecol. Syst. 10: 1-12.

Yanchinski, S. 1986. Plant engineered to kill insects. New Scientist 105: 25.


ADDITIVE EXPRESSION OF ADAPTIVE ALLELES
FREQUENCY OF ADAPTIVE ALLELES
1.0
0.8
0.6
0.4
0.2
0.0
0
10
20
30
GENERATION
1A


RECCESSIVE EXPRESSION OF ADAPTIVE ALLELES
FREQUENCY OF ADAPTIVE ALLELES
1.0
0.8
0.6
0.4
0.2
0.0
0
50
100
150
200
GENERATION
ONE HALF PLANTS
ALL PLANTS
1B